AN ABSTRACT OF THE THESIS OF

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Title: Removal of *E. coli* with Alternative Media Biosand Filters.

Abstract approved:

_____

Brian D. Wood

When Biosand filters cannot be constructed with crushed quarry rock due to resource limitations, a suitable alternative filter media is needed. In this research, two crushed quarry rock alternatives were examined. Three bench-scale Biosand filters with crushed rock, beach sand, and heat-treated beach sand media were simultaneously dosed with Willamette River water seeded with K-12 *E. coli* for 31 days. Influent and effluent filtrate was analyzed for *E. coli* using 3M Petrifilm *E. coli*/Coliform plate counts; influent and effluent pH, conductivity, turbidity, dissolved oxygen, and temperature were monitored. All three filters achieved stable *E. coli* removal efficiencies of 99% or greater after filter maturation, suggesting that it is possible to effectively use beach sand and heat-treated beach sand in Biosand filters for pathogenic bacteria removal. Mean effluent *E. coli* concentrations for crushed rock, beach sand, and heat-treated beach sand filters were 12, 29, and 30 CFU/mL respectively. Crushed rock filter effluent was significantly lower in mean effluent *E. coli* concentration than beach sand ($P < 0.001$) and heat-treated beach sand ($P < 0.001$)

filter effluents, suggesting that beach sand and heat-treated beach sand media should only be used as a secondary option to crushed rock media due to potentially greater exposure risk to pathogenic bacteria.

Removal of *E. coli* with Alternative Media Biosand Filters

by
Nathan J. Fulton

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Nathan J. Fulton, Author

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Dedicated to the memories of Jennifer Harvester and Elizabeth “Betty” Fulton.

Removal of *E. coli* with Alternative Media Biosand Filters

1 Introduction

Diarrheal disease is responsible for the deaths of 1.8 million people annually, 90% of whom are children under the age of 5. Over 1 billion people do not have access to improved water sources, which, along with inadequate sanitation and hygiene, are responsible for 88% of diarrheal disease. Household water treatment can reduce incidence of diarrhea by between 35% and 39% and improved water supply decreases diarrheal morbidity by between 6% and 25% (WHO 2004).

Intermittently operated slow sand filters, also known as Biosand filters (BSFs), are an inexpensive water treatment technology suitable for use in low income and rural communities and households. BSFs require minimal maintenance, significantly reduce the risk of waterborne disease, and are simple to construct and use. Despite the simplicity of BSFs, research is needed to better characterize the treatment mechanisms, and very few studies have explored the use of alternative filter media. Alternative filter media for BSFs may be needed, for example, in remote communities without access to crushed quarry rock. One potential BSF media alternative is beach sand, but concern remains over the potential efficacy of beach sand as an acceptable replacement substrate to be used in a Biosand filter (Manz 2007). This question is answered by comparing the removal efficiency of *E. coli* strain K-12 in three identical BSFs constructed with crushed rock filter media, untreated beach sand filter media, and heat-treated beach sand filter media of similar hydraulic conductivities, while

influent *E. coli* concentration, dosing volume, initial filtration rate, environmental conditions, and dosing frequency are the same for each filter.

2 Literature Review

2.1 History of Slow Sand Filtration

Earliest records of drinking water treatment date back to 4000 B.C., where Sanskrit and Greek text mention the aesthetic improvement of drinking water by means of charcoal filtration, straining, sunlight exposure, and boiling. Around 1500 B.C., chemical alum was first used by the Egyptians to clarify water by flocculation and sedimentation. 3300 years after Egypt's discovery of alum clarification, slow sand filtration finally surfaced as a common method of drinking water treatment (EPA 2000).

The first recorded slow sand filter for water treatment was experimentally developed by John Gibb in 1804 for his bleachery in Paisley, Scotland, where surplus treated water was sold to the public. Practical details of slow sand filtration were improved upon by Gibb and others, which resulted in the installation of a slow sand filtration system in London for the Chelsea Water Company in 1829 by James Simpson. The advantages of water filtration were so evident in aesthetic water quality that all water collected from the River Thames within 5 miles of St Paul's Cathedral was to be

filtered prior to public distribution under the Metropolis Water Act of 1852 (Huisman and Wood 1974).

Although water filtration was seen as advantageous to water quality during the mid-1800's, water quality was primarily determined by visual means; filters were simply viewed as a mechanical method of removing turbidity and suspended solids (Huisman and Wood 1974). In 1855, epidemiologist John Snow monitored a cholera outbreak in London. Dr. Snow determined that the disease was caused by consumption of water from a public well that had been contaminated by sewage. By the end of the 1800's, Louis Pasteur had established "germ theory", which described the transmission of disease by microorganisms via water and other media. Such discoveries shifted the focus of drinking water quality toward pathogens and methods of contamination removal (EPA 2000).

The effectiveness of pathogen removal by sand filtration was demonstrated by another cholera epidemic along the River Elbe in 1892, where neighboring cities of Hamburg and Altona both drew their drinking water. Hamburg distributed water without filtration, while down-stream Altona filtered its entire water supply. Cholera infections in Hamburg caused the death of 1.3% of its population, while Altona largely avoided the epidemic with less than 0.3% drop in population due to cholera. Many of

the deaths in Altona were suspected to have occurred as a result of infection while visiting neighboring Hamburg (Gainey and Lord 1952).

The first slow sand filters in the United States were installed in 1885, and in 1899, the first automatic pressure filters were patented in England. Various improvements, modifications, and variations of the slow sand filter have since been developed, but these changes have primarily focused on the construction, operation, and control of the filter (Huisman and Wood 1974).

2.2 Biosand Filtration and Traditional Slow Sand Filtration

The Biosand filter is a point-of-use (POU) water treatment technology developed in the 1990's by Dr. David Manz, who hypothesized that the primary component responsible for contaminant removal in slow sand filters is the biological layer, known as the *Schmutzdecke* (Buzunis 1995). However, little direct evidence of significant removal of contaminates by biological mechanisms in Biosand filters is present in literature, with the exception of virus removal (Elliott, DiGiano, and Sobsey 2011). Unlike traditional slow sand filters (TSSF) which are continuously operated, the Biosand filter was designed to be intermittently operated by the use of a swan-necked effluent pipe which maintains a resting supernatant depth of approximately 5 cm,

allowing for the diffusion of oxygen to the *Schmutzdecke* while the filter is not in use. The ability of the BSF to operate intermittently eliminates the necessity of engineered water supply, distribution, and storage systems that are cost-prohibitive in many situations. The filter bed depth of BSFs is smaller than it is for TSSFs, ranging from 0.8 m to 2 m in total height, where TSSFs are 3.5 m to 4 m. Another important difference between TSSFs and BSFs is the cleaning and maintenance process; the surface media of BSFs are gently agitated by hand and a significant portion of the supernatant is removed such that no filter media is lost. TSSFs use a system of harrowing (raking the filter surface) and scraping (removing media from the filter surface) to maintain acceptable filtration rates (Manz 2008).

2.3 BSF Performance Comparison with Other Point-of-Use Water Treatment Technologies

The majority of Biosand filter research to date has assessed filtration performance by one of three general methods: recording diarrheal disease reduction after BSF implementation in households, measuring the removal and inactivation of indicator organisms, and measuring the removal and inactivation of indicator viruses. Research on the removal of arsenic and indicator viruses with iron-amended BSFs has also been conducted.

Field studies that recorded instances of diarrheal disease amongst communities where BSFs were implemented have shown approximately 40 to 60 % reduction of diarrheal disease (Stauber, Ortiz, and Sobsey 2007; Stauber, Ortiz, et al. 2009; Stauber, Fabiszewski, et al. 2009; Jenkins, Tiwari, and Darby 2011). During the initial 6 months of BSF implementation in Bonao (Dominican Republic), households with BSFs reported 45% less diarrheal disease than households without BSFs (Stauber, Ortiz, and Sobsey 2007). Further study of BSF implementation in Bonao showed that incidents of diarrheal disease in households with BSFs was 53 % of incidents recorded in control households (Stauber, Ortiz, et al. 2009). In Ghana and Cambodia, a case study showed that households with BSFs reduce cases of diarrheal disease by 60% (Stauber, Fabiszewski, et al. 2009).

Reduction of incidents of diarrheal disease is particularly important because 90% of diarrheal disease related deaths occur in children under the age of 5 (WHO 2004). Along the River Njoro (Kenya), mothers of households were asked how many days their children (less than or equal to 15 years of age, with at least one child 4 years old or younger) had diarrhea on a weekly basis. The 30 households with BSFs reported that children spent 2.0% of days with diarrhea while the 29 households in the control group reported 5.2% (Tiwari et al. 2009).

Although recording instances of diarrheal disease is the most direct method of determining the impact of the implementation of a technology, all of the formerly discussed studies lack methodology to determine any biased over- or under-reporting of diarrheal episodes.

Indicator organisms, like *E. coli*, have been used to assess the performance of BSFs in both controlled laboratory experiments and in-the-field assessments. Membrane filtration methods have frequently been used for the enumeration of indicator bacteria in both situations (Buzunis 1995; Palmateer et al. 1999; Stauber et al. 2006; Elliott et al. 2008; Jenkins, Tiwari, and Darby 2011). Previous field studies in Bonao (Dominican Republic), and along the River Njoro (Kenya), have shown greater than 80% mean reduction of indicator bacteria in BSFs and 50-66% lower mean indicator bacteria concentrations in drinking water of households with BSFs when compared to households without BSFs (Stauber et al. 2006; Stauber, Ortiz, et al. 2009; Jenkins, Tiwari, and Darby 2011).

Laboratory studies of BSFs frequently report greater than 90% reductions of indicator bacteria (Buzunis 1995; Stauber et al. 2006; Elliott et al. 2008; Jenkins, Tiwari, and Darby 2011) and 99.98% removal of Cryptosporidium oocysts and 100% removal of Giardia cysts in BSFs has been observed (Palmateer et al. 1999). However, variables

such as pause time between filter dosing, volume of the dosing, influent water characteristics, and duration of filter operation can influence indicator bacteria removal efficiency. Like traditional slow sand filters, BSFs require a maturation period to achieve high removal efficiencies; however the duration of this period may differ due to varying influent water properties, dosing regimens, operator behavior, filter media properties, and environmental conditions (Buzunis 1995; Baumgartner, Murcott, and Ezzati 2007; Elliott et al. 2008; Kubare and Haarhoff 2010). Although many operational observations have been made in literature regarding dosing volume, pause time between dosing, and their relation to indicator bacteria removal efficiency, further research is needed to determine relationships to describe the optimum operational parameters for a particular household's water volume requirement (Baumgartner, Murcott, and Ezzati 2007).

Indicator viruses have been used to assess the BSF for the removal of pathogenic viruses and have been conducted with Echovirus type 12, PRD-1, and MS-2 viruses. Echovirus type 12 exhibited removals in BSFs ranging from 1 log unit to greater than 3 log units (90% to greater than 99.9%), while the geometric mean of reduction after 30 days was 2.1 log units (99.2%) (Elliott et al. 2008).

Removal of bacteriophages MS2 and PRD-1 have ranged from 0 log to 1.3 log units (0 to 95%), while geometric mean of reduction has been reported at 0.5 log units (70%) (Elliott et al. 2008). Due to differences in removal between Echovirus type 12 and Bacteriophages, virus reduction in BSFs may be dependent on the specific virus (Elliott et al. 2008). Continued research on MS2 and PRD-1 reduction in BSFs showed that reduction did not occur when microbial activity was inhibited by sodium azide, where first order reduction of MS2 and PRD-1 normally occurred during the idle time in BSFs (Elliott, DiGiano, and Sobsey 2011).

Iron amended BSFs have also shown promise in removal of viruses and arsenic from drinking water. Over 20 pore volumes of dosing water, a sand-only column exhibited 0.5 log units removal of MS2 bacteriophage, where an iron-amended sand column removed 5 log units, and iron amended BSFs performed above 4 log units removal (Bradley et al. 2011). However, influent phosphate concentrations greater than 0.5 mg/L and iron concentrations less than 5 mg/L have been observed to hinder arsenic removal in amended BSFs (Chiew et al. 2009).

Point-of-use water treatment is not limited to slow sand filtration; solar disinfection (SODIS), chlorination and safe storage, chlorination and coagulation, and ceramic filtration are other well documented methods. These water treatment methods have

been compared and evaluated by sustainability criteria, including water quantity, water quality, ease of use, cost, and required supply chain. Each water treatment method was rated on a scale of 1 to 3 for each sustainability criteria. BSFs were determined to be the most sustainable technology, closely followed by ceramic filters. Although more expensive to initially implement than ceramic filters, BSFs are capable of producing a larger quantity of water and require practically no supply chain after implementation due to the greater durability of BSFs (Sobsey et al. 2008).

In a meta-regression study which compared various POU water treatment technologies, ceramic filters were determined to be the most effective technology for the prevention of waterborne disease over the duration of 52 weeks. However, only three un-blinded studies of Biosand filters have been included in the meta-regression, none of which exceeded 26 weeks of data, suggesting that at least one large-scale, preferably blind, 52 week-long study is needed to properly assess the disease risk with the implementation of Biosand filters (Hunter 2009). Nonetheless, the meta-regression study indicates that chlorination, SODIS, and chlorination with coagulation (disinfection methods) have little or no health benefit over a 12 month period when implemented in developing country situations (Hunter 2009).

The failure of disinfection methods to reduce disease risk may be linked to the decrease in post-implementation compliance (Rainey and Harding 2005; Sobsey et al. 2008; Hunter 2009). Compliance with BSFs is greater than 85% and ceramic filters can be as high as 88% post-implementation, but ceramic filters are prone to breakage and subsequential abandonment (Sobsey et al. 2008). Although post-implementation compliance may be initially higher for ceramic filters, the more durable BSFs are arguably a superior long-term solution for POU water treatment.

Biosand filters depend on the availability of crushed quarry rock, which in some locations may not be available, or the material cost may be unaffordable. When such situations occur, the implementer of the BSF technology has historically replaced the crushed quarry rock with the best available media, including, but not limited to aggregate and sand from rivers and beaches (Manz 2007). However, research investigating the use of alternative filter media in BSFs is lacking, with the exception of iron-amended BSFs. In order to identify the best alternative media sources and media preparation practices, BSFs with various types of media and preparation practices need to be evaluated and compared to traditional BSFs using crushed quarry rock.

2.4 Construction and Maintenance of Biosand Filters

Biosand filters can be dissected into four main components: the filter housing, the diffuser, the effluent pipe, and the filter media. The filter housing is constructed of either plastic or concrete and is approximately 1 m tall. The filter surface area varies between different models of BSFs, but they are typically around 0.06 m^2 based on concrete mold diagrams typically used for construction (Manz 2010). The filter media consists of a minimum of three unique layers of material: (1) underdrain gravel, (2) a separation layer of small gravel, and (3) a thick layer of filtration sand over the separation layer (called the filter bed). All three of these layers are traditionally obtained from crushed quarry rock. Figure 2.1 shows the typical components of a BSF.

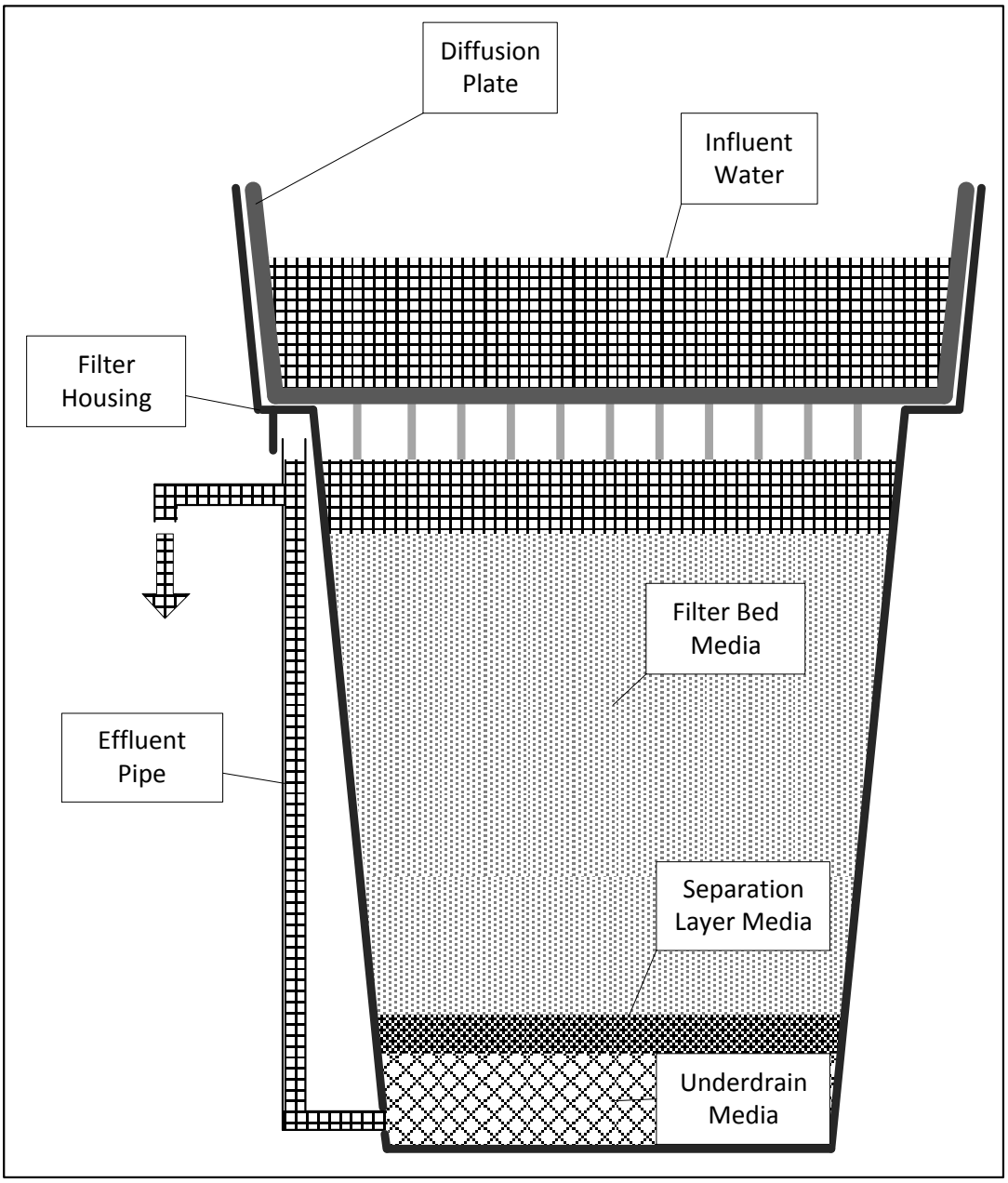

Figure 2.1: Biosand filter components.

After the filter media is obtained, it is sorted by size with sieves, and is repeatedly hand-washed in small quantities with water. The underdrain media is added to the filter housing, followed by enough water to cover the previous media layer by approximately 20 cm. The separation layer is added, followed by multiple layers of filtration sand until the media surface is 5 cm below the resting water level. The purpose of adding filter media into a small depth of water is to prevent air pockets and preferential flow pathways within the filter bed. The filter is then flushed with approximately 60 L to 100 L of water until the turbidity of the effluent is less than that of the influent.

After flushing, the filter is cleaned by adding water to the filter, removing the diffuser, gently agitating the surface of the filter no more than 1 cm deep, and decanting most of the supernatant. This cleaning technique is also used after commissioning when the flowrate has decreased to unacceptable levels. After cleaning, the filter is calibrated to have a maximum filtration rate of 600 L/hr/m^2 by removing, washing, and replacing filter media to increase flowrate, or by removing and replacing filter media with less-washed filter media to decrease flowrate. Sodium hypochlorite disinfection solution is prepared by adding perfume-free household bleach to filtered water until a distinct chlorine smell is detected after the solution is well stirred (Manz 2010). Disinfection of the filter is accomplished by adding approximately 2 L of sodium hypochlorite

solution through the effluent pipe and into the underdrain and separation layers of the filter. After 20 minutes of disinfection, the filter is flushed again until chlorine taste and odor is no longer detectable. Cleaning of the filter is conducted as needed (Manz 2009; Manz 2010).

2.5 Mechanisms of Particulate Removal

The predominant mechanisms of particulate removal in slow sand filters can be divided into mechanical and biological mechanisms. Mechanical removal mechanisms can be sub-divided into transport mechanisms and attachment mechanisms. Transport mechanisms are responsible for the collision of particles to the filter media, while attachment mechanisms bind the particles to the filter media. Transport mechanisms include sedimentation, straining, interception, inertial, and hydrodynamic effects, and Brownian motion (Hendricks, Barrett, and AWWA Research Foundation. 1991; American Water Works Association and Letterman 1999). Each mechanism is described briefly in the following.

2.5.1 Sedimentation

Sedimentation is the result of gravity acting upon a particle with greater density than the surrounding fluid (Hendricks, Barrett, and AWWA Research Foundation. 1991). Because the force of gravity is greater than the force of buoyancy, the particle is transported downward until it has settled onto the solid filter media. Figure 2.2 shows sedimentation within a filter bed while the fluid is stagnant, representing the time periods between each dosing of influent water.

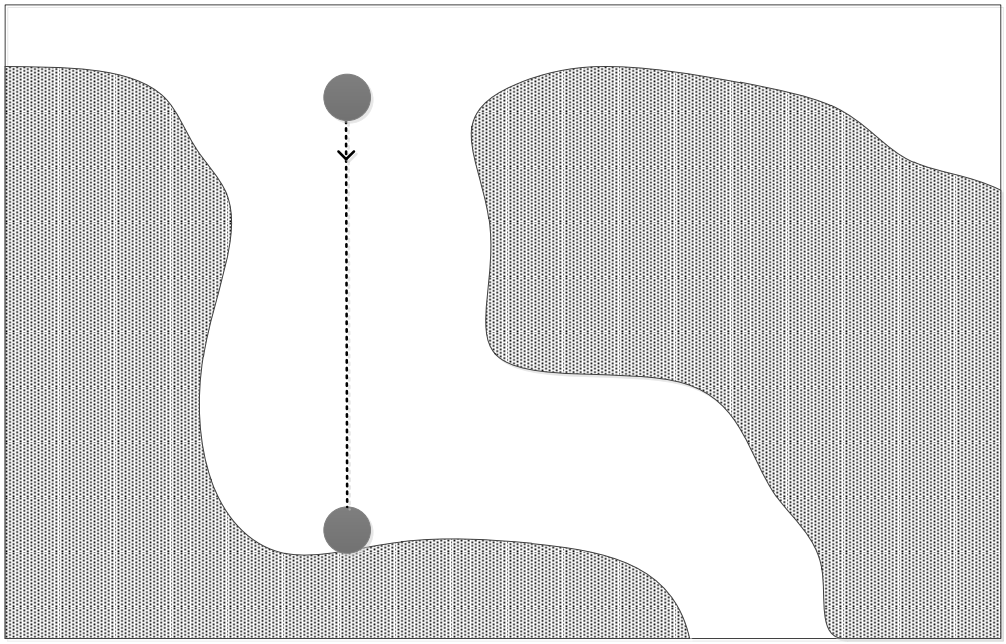

Figure 2.2: Sedimentation without fluid flow.

Because the fluid is stagnant, the gravitational, buoyant, and drag forces are coaxial. The gravitational force is initially greater than the sum of the buoyant force and drag force, thus the particle accelerates downward. However, terminal velocity is assumed to be reached instantaneously in water treatment systems, thus the transient state can be neglected. Assuming laminar flow conditions, Stokes' equation for laminar flow (*Eq. 2.1*) can be used to determine the settling velocity (American Water Works Association and Letterman 1999).

$$v_t = \frac{g(\rho_p - \rho_f)d^2}{18\mu} \qquad (2.1)$$

Where v_t = terminal settling velocity

g = gravitational constant of acceleration

ρ_p = particle density

ρ_f = gravitational constant of acceleration

d = particle diameter

μ = absolute (dynamic) liquid viscosity

Figure 2.3 demonstrates the sedimentation of a suspended particle with fluid passing through a filter bed and is representative of a Biosand filter during active dosing of influent water. The deviation of a particle (dashed line) from the fluid flow streamlines (solid line) due to sedimentation is displayed.

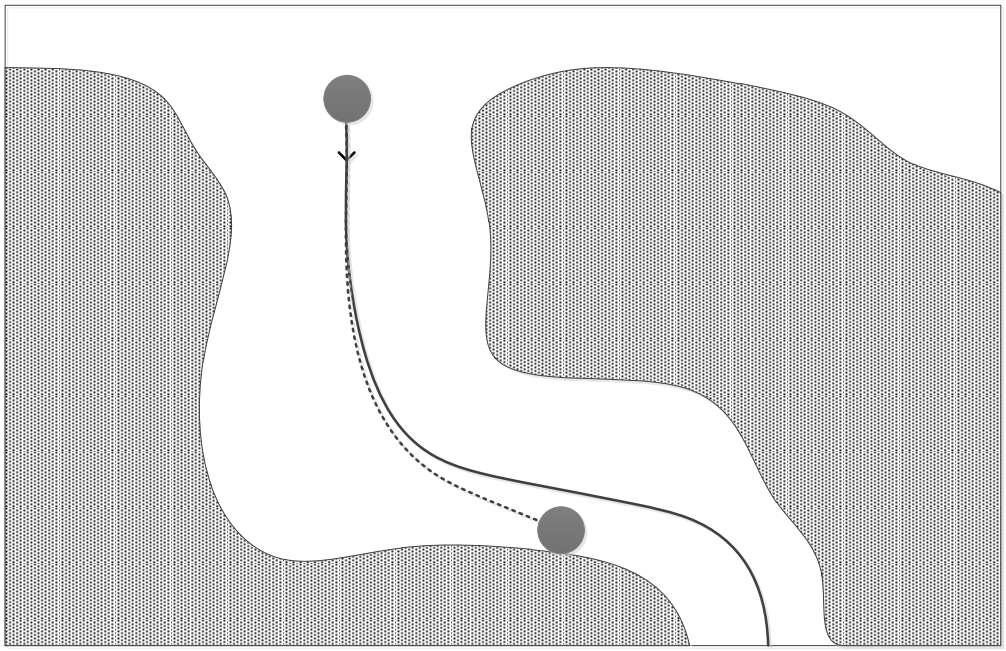

Figure 2.3: Sedimentation with fluid flow. Dotted line represents particle path while the solid line represents the fluid streamline originating at the initial particle position.

Particles transported along streamlines within the mobile fluid can be removed with the aid of sedimentation, but efficiency of removal by sedimentation can be expected

to decrease with increasing fluid flow due to dependencies on residence time and hydrodynamic conditions.

2.5.2 Straining

Particles larger than the pore spaces of the filter bed are removed at the filter/supernatant interface. With use of the filter, removed particles accumulate at the filter/supernatant interface and within the filter pore space, resulting in a reduced effective pore size and increased removal efficiency. However, the effective pore size eventually decreases to the point where hydraulic conductivity is too low to efficiently pass water and the filter surface is cleaned to increase hydraulic conductivity at the cost of particle removal efficiency (Buzunis 1995). Figure 2.4 illustrates the straining mechanism.

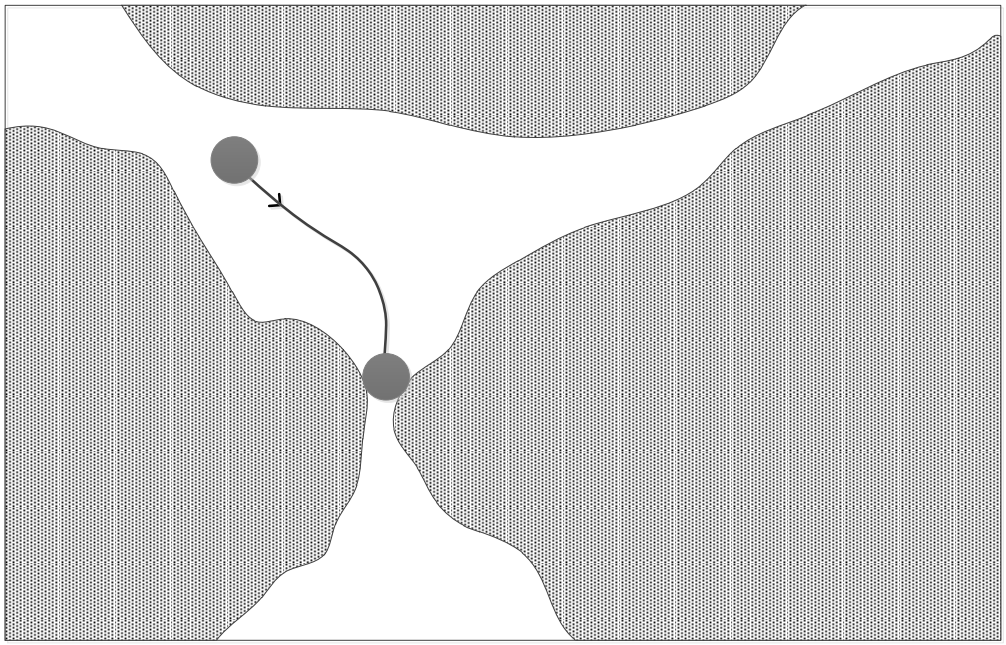

Figure 2.4: Straining.

Figure 2.4 depicts a particle traveling along a streamline (solid line) until the particle is removed from suspension because the pore diameter is smaller than the particle diameter.

2.5.3 Interception

Particles smaller than filter bed pores can be transported through the filter bed while traveling along streamlines. However, particles traveling along streamlines which are less than one particle radius away from the pore wall will cause the particle to collide

with the pore wall, as depicted in Fig. 2.5 (Hendricks, Barrett, and AWWA Research Foundation. 1991).

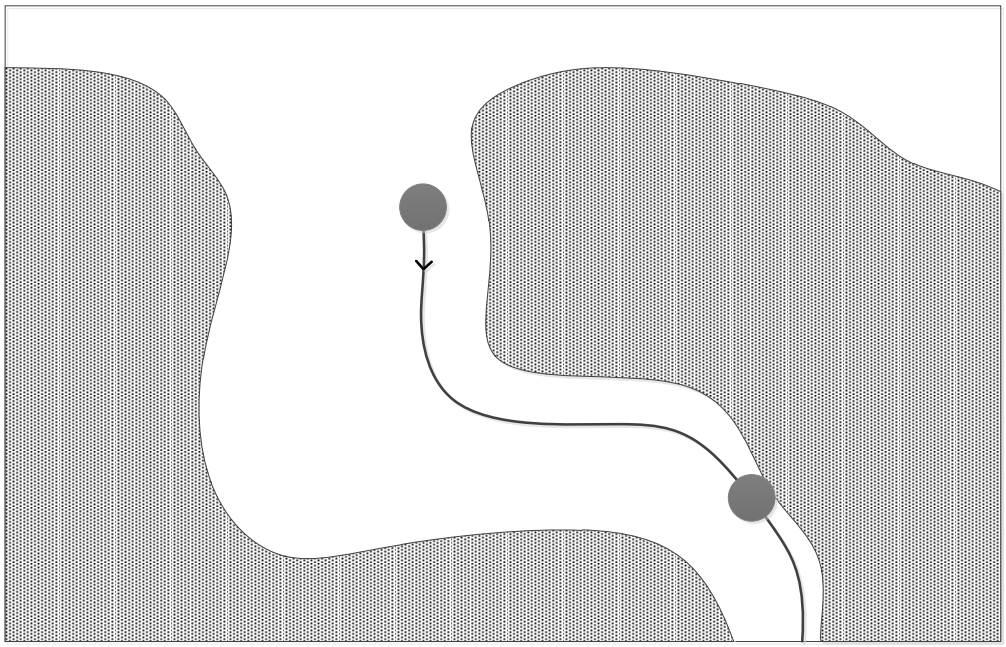

Figure 2.5: Interception.

Figure 2.5 shows a particle following a streamline (solid line) that comes into one particle radius of the pore wall and is intercepted.

2.5.4 Inertial and Hydrodynamic Effects

Flow pathways through filter beds are very tortuous, thus a particle suspended in the mobile fluid can collide with the pore wall due to the particle's inertia. Figure 2.6 shows a particle with an initial velocity along the fluid streamline that collides with the pore wall due to inertia.

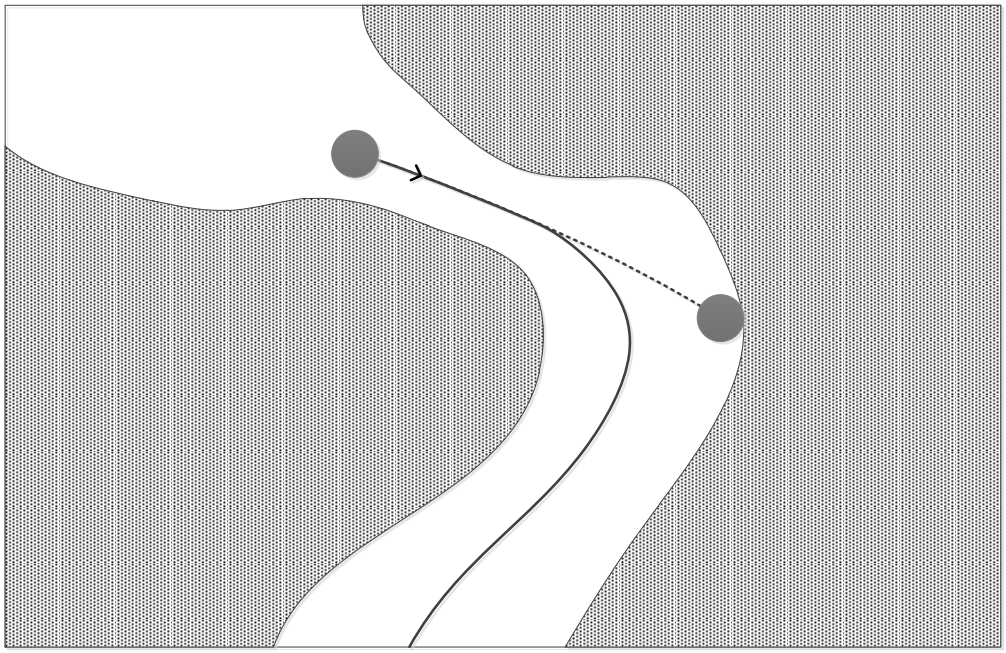

Figure 2.6: Inertial effect. Dotted line represents particle path while the solid line represents the fluid streamline originating at the initial particle position.

When the particle approaches the channel bend, inertia causes the particle to travel across streamlines and may cause a collision with the pore wall. However, the initial position of the particle, hydrodynamic properties, and drag forces caused by fluid flow relative to the particle may prevent collision and keep the particle in suspension (Hendricks, Barrett, and AWWA Research Foundation. 1991; American Water Works Association and Letterman 1999).

2.5.5 Brownian Motion

Brownian motion can be described as the random movement of a particle within a fluid due to molecular collisions. When the random movement of the particle causes a collision between the particle and the filter bed, the particle may be removed from suspension. The frequency of molecular collision is relative to the thermal energy of the fluid, thus particle removal efficiency by Brownian motion rises with an increase in fluid temperature (Hendricks, Barrett, and AWWA Research Foundation. 1991). Particles smaller than approximately 1 μm are typically affected by Brownian motion (Yao, Habibian, and O'Melia 1971). Figure 2.7 illustrates the collision of a particle and pore wall in a filter bed by Brownian motion.

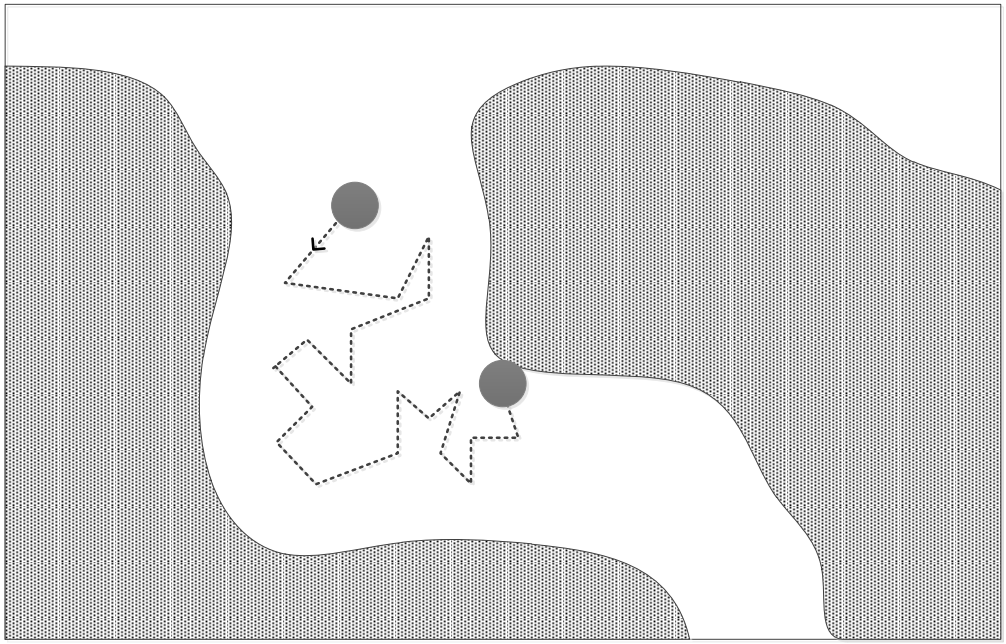

Figure 2.7: Brownian Motion.

2.5.6 Attachment Mechanisms

Van der Waals forces are the main attractive short-range forces responsible for attachment of particles to the filter bed. However, other short-range forces known as electrostatic repulsive forces must be overcome in order to achieve attachment. Van der Waals forces are dominant over short distances between two particle surfaces, while electrostatic repulsive forces are dominant at greater distances when the bulk solution has a low ionic strength. At high ionic strength, electrostatic repulsive forces are reduced, and attractive forces can become dominant at greater separation distances

(American Water Works Association and Letterman 1999). Figure 2.8 shows two cases of attractive, repulsive, and net interaction energies between two particles in low (a) and high (b) ionic strength solutions.

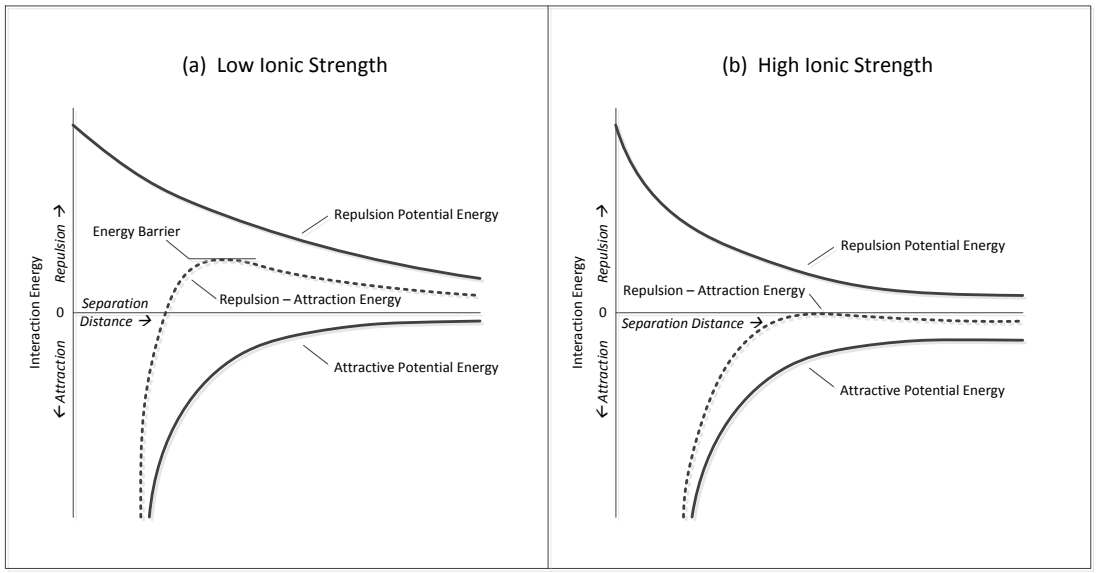

Figure 2.8: Short-range interaction energies between particles at low and high ionic strength. This figure was adapted from *Water quality and treatment: a handbook of community water supplies* (American Water Works Association and Letterman 1999).

In filtration systems with low ionic strength solutions, as in Fig. 2.8(a), transport mechanisms can cause suspended particles to acquire momentum toward the filter bed particles. If the mobile particle's kinetic energy is great enough, the energy barrier can be overcome and the mobile particle will adhere to the filter bed particle. As ionic strength increases, the energy barrier decreases, suggesting that less kinetic energy is

needed for attachment, resulting in higher attachment efficiency. In high ionic strength solutions, as in Fig. 2.8(b), no energy barrier must be overcome, indicating that a particle must only be transported within the effective region of short-range forces. At ionic strengths between conceptual cases (a) and (b), an energy barrier would exist, but it would be less than that of case (a).

2.5.7 Biological Mechanisms of Particulate Removal

Biological mechanisms of particulate removal in slow sand filtration include predation, biological oxidation, death, and inactivation (Haarhoff and Cleasby 1991; Weber-Shirk and Dick 1997). Predation, also known as bacterivory or grazing, is considered the primary biological particle removal mechanism in slow sand filters (Weber-Shirk and Dick 1997) and is defined as the consumption of particles by microbes. Experiments by Weber-Shirk and Dick (1997) involved the reversible inhibition of predation in slow sand filters by blocking oxidative phosphorylation with sodium azide. These experiments revealed that only particles less than 2 μm were statistically affected by biological activity (Weber-Shirk and Dick 1997). Previous research on predation indicated that freshwater flagellated protozoa primarily graze upon bacteria with 1.1-1.3 μm cell diameters, while decreasing consumption efficiency was observed of cells with greater or lesser diameters (Chrzanowski and

Simek 1990); this was consistent with the observations of Weber-Shirk and Dick (1997). In 2004, a similar study inhibited *Aeromonas hydrophila* with cycloheximide while measuring filter effluent with heterotrophic plate counts and total bacterial counts. Results from cycloheximide inhibition experiments confirmed that predation is an important removal mechanism for bacteria (Bomo et al. 2004).

3 Materials and Methods

3.1 Apparatus

Filter bodies were made of 9.7 cm I.D., 13 cm O.D., 66 cm long cast nylon 6 pipe with a 1.3 cm thick nylon base plate welded to the bottom of the nylon pipe. Quarter inch schedule 40 PVC pipe was used to construct the effluent pipe and the anti-siphon pipe. As depicted in Fig. 3.1, the 90° bend of the effluent pipe and the 180° bend of the anti-siphon pipe were heat-formed to shape. The effluent pipe was installed into the filter housing with the center of the pipe 4.7 cm above the bottom edge of the cast nylon tube.

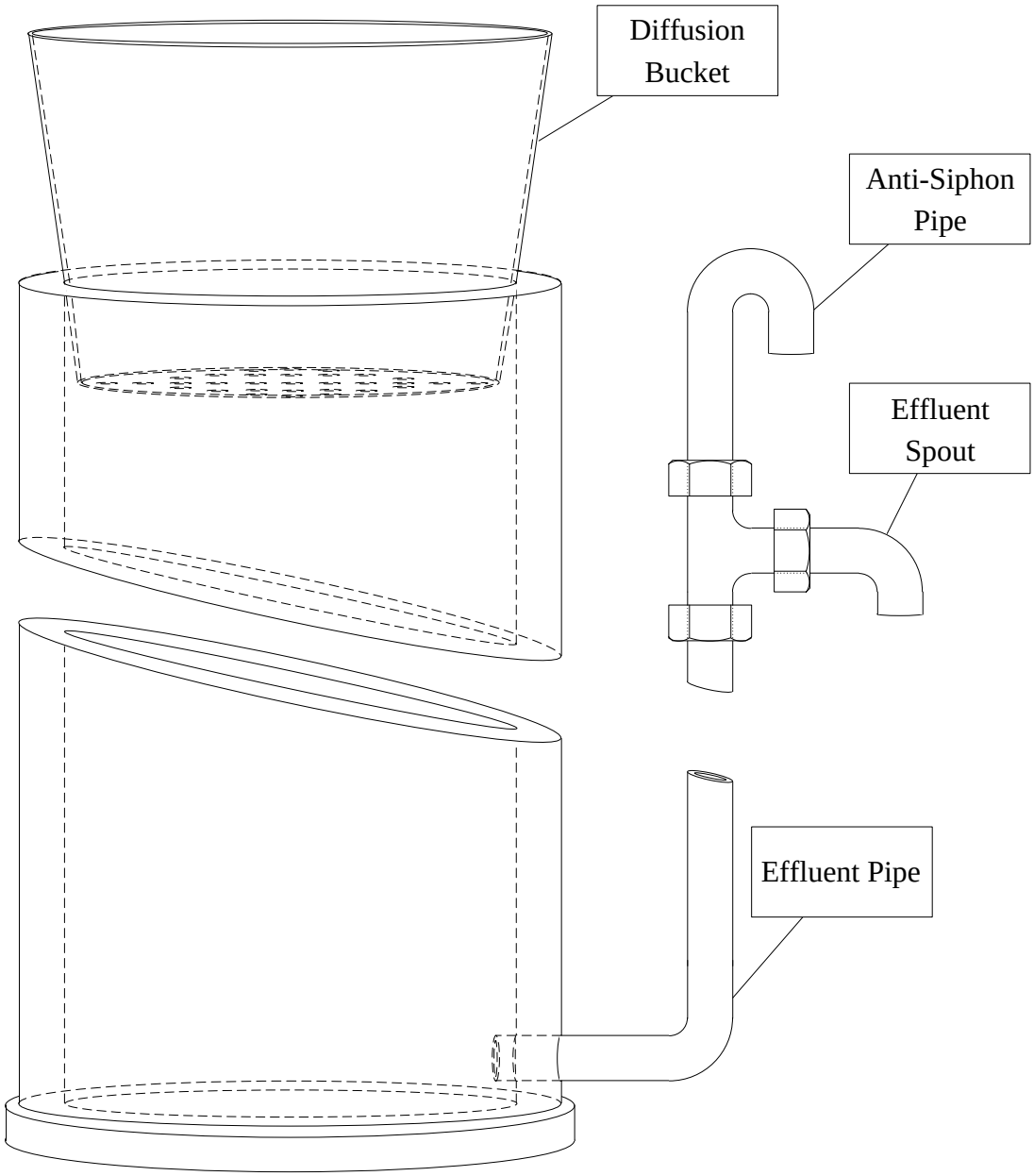

Figure 3.1: Diagram of BSF filter housing apparatus.

The 180° bend on the anti-siphon pipe was implemented to prevent contamination of water in the effluent pipe while remaining open to atmospheric pressure. A dispersion plate was constructed with a 1 L food grade bucket with 3 cm holes drilled in a 2.5 cm spaced grid pattern on the bottom of the bucket, as described in the standard BSF construction procedures (Manz 2010).

Crushed rock filter media was sourced from Coffin Butte quarry, north of Corvallis, Oregon. The crushed rock filter media was sorted through 12.7 mm, 6.4 mm, and 2.4 mm sieves to produce underdrain media of 6.4 mm to 12.7 mm, separation layer media of 2.4 mm to 6.4 mm, and filter bed media of less than 2.4 mm diameter respectively. Crushed rock underdrain and separation layer media was used in all three BSF columns, while only one BSF contained crushed rock filter bed media. The other two BSFs contained beach sand and heat treated beach sand from Fogarty Beach, Oregon (latitude: 44.840, longitude: -124.052). Fogarty Beach sand was selected due to its similar hydraulic conductivity to the crushed rock filter bed media. Beach sand was collected from a depth greater than 20 cm below ground surface and was sorted through a No. 8 sieve to produce filter bed media ($d < 2.4$ mm). All media was washed according to standard BSF construction procedures (Manz 2010). After the media was washed, heat-treated beach sand media was produced from sorted Fogarty

Beach sand that was heated in a Thermolyne F-A1730 muffle furnace at 550°C for 5 hours.

A known volume of water was added into each filter body (approximately 5 cm deep) followed by 8 cm of underdrain media, 3 cm of separation media, and 40 cm of filter bed media. All filter media was added in increments of approximately, but not greater than 5 cm layers, while an approximate 5 cm head of water was maintained above the filter surface by adding known volumes of water to each filter between additions of media. Resting system fluid volume was determined by subtracting the volume of displaced water from the total volume of water added to each filter during the media addition process. The volume of water withheld by the effluent pipe was estimated to be approximately 40 mL, and approximately 220 mL held above the filter media surface.

To sterilize the effluent pipe, underdrain layer, and separation layer, 350 mL of 0.6% sodium hypochlorite solution was added to the bottom of the filter through the effluent pipe. Each filter was then purged by filtering 15 L of raw Willamette River water collected from the H.D. Taylor Water Treatment Plant in Corvallis, Oregon. While filters were purged, filtration rate was calibrated to an initial surface loading rate of approximately 600 $L/(h \cdot m^2)$ at a head of 13 cm by washing or replacing filter media to

achieve a lesser or greater fines content, as is described by standard BSF construction procedures (Manz 2010).

3.2 Source Water, E. coli Seeding and Filter Dosing

Tryptic soy broth solution at a concentration of 8 g/L was used to culture K-12 *E. coli* on an incubated shaker table at 35°C and 200 rpm for 4 hours, then was immediately refrigerated at 4°C as described in *Appendix A.* Culturing was performed weekly from frozen K-12 *E. coli* stock. During filter operation, 40 L of raw Willamette River water was collected in two autoclaved 25 L Nalgene carboys every other day from H.D. Taylor Water Treatment Plant in Corvallis, Oregon. Each carboy contained 20 L of raw river water and was allowed at least 24 hours of storage in the lab for temperature stabilization. Each day, one carboy with 20 L of temperature stabilized river water was seeded with 2 mL of serially diluted K-12 *E. coli* culture at 10^{-2} and 10^{-1} dilutions. These dilutions were chosen to achieve target *E. coli* dosing concentrations of approximately 300 CFU/mL for days 0 to 13 and 3000 CFU/mL for days 14 to 31.

Each filter was dosed daily (12:00 P.M.) with 4L of seeded feed water in 1 L increments. The second liter of seeded feed water was not added to any filter until

sampling was completed, and the maximum pressure head on the filter was never allowed to exceed 13 cm.

Prior to filter dosing experiments, 500 mL samples were collected from Taylor Water Treatment Plant in autoclaved 500 mL Nalgene bottles approximately every two days over the course of two weeks (7 samples). Samples were analyzed for turbidity and *E. coli*/coliform counts using 3M Petrifilm *E. coli*/Coliform count plates (n=3). This data was collected to establish an understanding of baseline characteristics of the Willamette River source water in order to improve experiment design.

3.3 Sampling

After seeding and mixing the feed water and prior to filter dosing, one 100 mL sample of feed water was collected in a 150 mL Erlenmeyer flask and was immediately tested for dissolved oxygen and temperature, followed by conductivity, pH, and turbidity. On every odd day of filter operation, five 1 mL samples were collected from the seeded feed water after mixing, were diluted by a factor of 10^{-1} (when target influent *E. coli* concentration was 3000 CFU/mL) as described in *Appendix B*, and were directly plated onto 3M Petrifilm *E. coli*/Coliform count plates according to manufacturer protocol.

After a given filter had passed 100 mL of effluent to purge the effluent tube, the following 100 mL of effluent was collected in a 150 mL Erlenmeyer flask and was immediately tested for dissolved oxygen and temperature followed by conductivity, pH, and turbidity. On every even day of filter operation, 500 mL of filtrate from each BSF was collected in 1 L sterilized bottles immediately after the first 200 mL of filtrate. Each filter was timed for the passing of the first 300 mL after the initial 100 mL purge volume to obtain a rough hydraulic conductivity estimate for each filter bed by the falling head permeability test described in *Eq. 3.1*. The first 100 mL purge volume was omitted from timing for the falling head permeability test to allow enough time for water to pass through the diffuser during a 1 L filter dosing.

$$k = \frac{aL}{At} * \ln\left(\frac{h_0}{h_1}\right) \qquad (3.1)$$

Where k = Hydraulic conductivity

a = Area of the cylinder where the falling head occurs

L = Soil column length

A = Soil column area

h_0 = Initial water height

h_1 = Final water height

t = Time duration of head drop from initial to final water height

The falling head occurs in the same cylinder as the soil column, thus the associated area values *a* and *A* are equivalent and cancel out. Because the initial water height h_0 and final water height h_1 both occur in the same diameter column, both can be multiplied by the area of the column to use initial and final volume of the head instead of height. This yields *Eq. 3.2*.

$$k = \frac{L}{t} * \ln\left(\frac{V_0}{V_1}\right) \qquad (3.2)$$

Where V_0 = Volume of water in initial head

V_1 = Volume of water in final head

With this equation, hydraulic conductivity can be estimated by measuring the time for the filter to pass the first 300 mL after a 100 mL purge volume from a 1 L filter dosing. Thus V_0 and V_1 are 900 mL and 600 mL respectively for this experiment.

After sample collection, each 1 L bottle with collected influent or effluent was mixed and five 1 mL samples from each sample collection bottle were directly plated onto 3M Petrifilm *E. coli*/Coliform Count Plates and incubated according to manufacturer instructions.

3.4 Analysis

3.4.1 E. coli and Coliform Enumeration

Filter influent and effluent concentrations of viable *E. coli* were measured with 3M Petrifilm *E. coli*/Coliform (E/C) count plates according to manufacturer instructions. Chromogenic compound BCIG (5-bromo-4-chloro-3-indolyl-beta-D-glucuronide) is present in Petrifilm E/C plates and is cut with enzyme beta-glucuronidase, resulting in a blue precipitate that dyes the colony. Beta-glucuronidase is produced by 94 to 96 % of all *E. coli* strains (Manafi 2000). Violet Red Bile (VRB) with lactose is also present in the plates, which promotes coliform growth and allows for the confirmation of lactose-fermenting bacteria in Petrifilm E/C plates by trapped gas underneath the film plate covers.

Five 1 mL samples were collected from each of the four monitored streams (influent, crushed rock filter effluent, untreated beach sand effluent, and heat-treated beach sand effluent). Petrifilm plates were incubated at 35°C for 24 hours and were counted according to manufacturer instructions.

3.4.2 Total Organic Carbon of Filter Media

Total organic carbon (TOC) of the crushed rock media and beach sand media was determined by loss on ignition. Three samples of approximately 100 g of each raw media (crushed rock media and beach sand media after sorting and washing) were dried at 105°C until the change in mass was less than 0.5%. Samples were then combusted at 550°C until less than 0.5% change in mass was observed. Samples were transferred with tongs and were allowed to cool in desiccators after being dried or combusted until they could be safely weighed. Prior to sample drying and combustion, crucibles were combusted at 550°C until change in mass was less than 0.5%.

3.4.3 Dissolved Oxygen and Temperature

Dissolved oxygen (DO) and temperature were measured with a Hach sensION156 according to manufacturer protocol. The DO meter was calibrated daily with the saturated air method according to manufacturer protocol and was cleaned with deionized water between samples.

3.4.4 Turbidity

Turbidity was measured with a Hach 2100P Turbidimeter according to manufacturer instructions. The turbidity meter was calibrated daily with Formazin standards and the sample vial was cleaned with deionized water and dried between samples.

3.4.5 Conductivity and pH

Conductivity and pH were measured according to manufacturer instructions on a VWR SR 60IC meter with a VWR SympHony two-cell epoxy platinum conductivity probe (14002-802) and VWR SympHony Waterproof Gel 3-in-1 pH electrode (14002-778). The conductivity and pH meter was calibrated daily with standard solutions. Between periods of use, the pH probe was stored in storage solution. Probes were cleaned with deionized water and gently dabbed dry between samples.

3.4.6 Chemical Oxygen Demand

Chemical oxygen demand (COD) of raw river water collected at Taylor Water Treatment Plant was assessed with a Hach DRB200 reactor/spectrophotometer and low-range (3-150 mg) COD reagent vials to determine approximate baseline COD

values for the source water according to Hach Method 8000. COD standard solutions (0 ppm, 50 ppm, and 100 ppm) were used to establish a standard curve to relate spectrophotometer readings to ppm COD.

3.4.7 Statistical Evaluation

Welch's T-Test was used for determining if significant differences exist between the means of two data sets. Welch's T-Test was chosen for its ability to assess two unpaired data sets with potentially different variances. *E. coli* concentrations, removal efficiencies, turbidity, conductivity, pH, hydraulic conductivity, and dissolved oxygen were all assessed using Welch's T-Test to determine significant differences between influent and effluent parameters, as well as differences between the crushed rock, beach sand, and heat-treated beach sand filters.

3.4.8 Analysis of Residence Time on E. coli Removal

On day 24 of the experiment, additional effluent samples were collected as described in Section 3.3 with the exception of collecting while the final 1 L of influent water was passing through each filter. Because of the 4 L total daily loading on each filter and the system fluid volume of 2 L, these samples are representative of filtrate that does

not rest in the filter during pause time and thus has a small residence time (t_R). This situation occurs when a single influent dose exceeds the system fluid volume. Samples were analyzed for *E. coli* concentration as described in Section 3.4.1 for exploring the effects of residence time on *E. coli* removal efficiency and effluent concentration.

4 Results

4.1 Baseline Willamette River Water Properties

Turbidity, *E. coli* and total coliform plate count data collected over two weeks from H.D. Taylor Water Treatment Plant are shown in Table 4-1.

Table 4-1: Willamette River water background turbidity, *E. coli* and total coliform plate counts.

Day	*E. coli* (CFU/mL)				Total Coliforms (CFU/mL)				Turbidity (NTU)			
	Plate 1	*Plate 2*	*Plate 3*	*Mean*	*Plate 1*	*Plate 2*	*Plate 3*	*Mean*	*Rep. 1*	*Rep. 2*	*Rep. 3*	*Mean*
1	0	0	0	< 1	5	4	3	4	5	5	5	5
3	0	0	1	< 1	4	6	6	5	3	3	3	3
5	2	0	0	< 1	14	8	7	10	2	2	2	2
7	5	0	0	2	23	18	15	19	5	5	5	5
9	1	0	0	< 1	7	6	6	6	4	4	3	4
12	0	0	0	< 1	1	4	3	3	3	3	2	3
13	0	0	0	< 1	0	1	1	< 1	2	3	2	2

Mean concentration of *E. coli* is less than 1 CFU/mL on all days except for day 7, where the largest plate count encountered for *E. coli* occurred at 5 CFU/mL. Mean total coliform plate counts ranged from < 1 to 19 CFU/mL with the maximum plate count occurring on day 7 at 23 CFU/mL. Considering the relative magnitude of the target influent concentrations, all observed background concentrations of *E. coli* fall

below 2 % of the 300 CFU/mL target influent concentration. Although all observed background concentrations of total coliforms falls within 8 % of the target influent concentration, the *E. coli* used for dosing is distinguishable from coliforms that are not *E. coli* on the Petrifilm E/C plates during BSF experimentation.

Willamette River water was collected on August 16, 2011 and was analyzed for Chemical oxygen demand (C.O.D.). All five replicates were less than the detectable concentration.

4.2 Total Organic Carbon of Filter Media

Loss-on-ignition procedures were used to determine approximate total organic carbon (TOC) content of source material for the BSFs. Collected data and results are summarized in Table 4-2.

Table 4-2: Total organic carbon analysis of source filter media by loss-on-ignition.

Sample I.D.	Crucible Mass (g)	Crucible + Sample Mass (g)	Crucible + Sample Mass (g) after 1st Drying	Crucible + Sample Mass (g) after 2nd Drying	% Change in Mass of Sample	Crucible + Sample Mass (g) after 3rd Drying	% Change in Mass of Sample	Crucible + Sample Mass (g) after 1st Combustion	Crucible + Sample Mass (g) after 2nd Combustion	% Change in Mass of Sample	% Organic Carbon by Mass	Mean % Carbon by Mass
Beach Sand-1	127.76	239.56	239.15	239.10	0.04%	239.10	0.00%	238.50	238.50	0.00%	0.54%	
Beach Sand-2	124.44	325.61	235.13	235.18	0.05%	235.16	0.02%	234.50	234.51	0.01%	0.59%	0.6%
Beach Sand-3	154.44	275.32	274.84	274.86	0.02%	274.86	0.00%	274.19	274.19	0.00%	0.56%	
Crushed Rock-1	137.21	248.00	238.08	237.95	0.13%	237.88	0.07%	236.88	236.90	0.02%	0.97%	
Crushed Rock-2	140.90	252.85	242.52	242.56	0.04%	242.40	0.16%	241.46	241.37	0.09%	1.01%	1.0%
Crushed Rock-3	136.48	250.90	240.08	240.12	0.04%	240.03	0.09%	239.01	238.95	0.06%	1.04%	

TOC analysis shows both crushed rock and beach sand source media as having less than or equal to 1% organic carbon.

4.3 Influent and Effluent Temperature

Influent and effluent water temperatures were recorded throughout the operation of the BSFs and are shown below in Fig. 4.1.

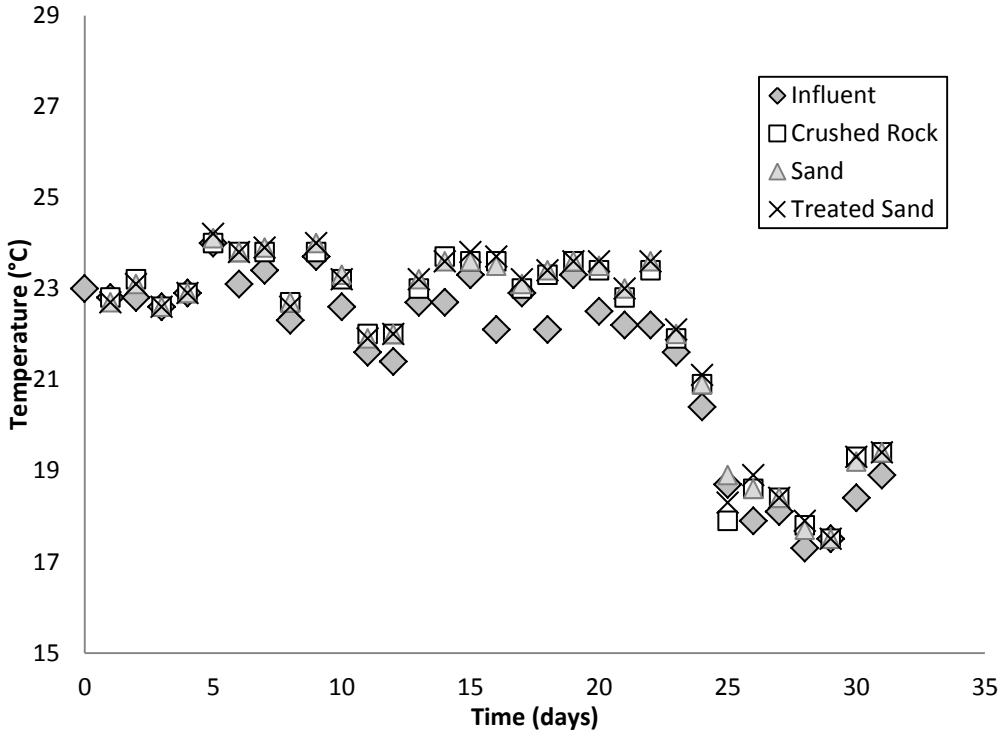

Figure 4.1: Biosand filter influent and effluent temperatures.

From filter start-up to day 22, influent and effluent temperatures remained between 21.4 °C to 24.2 °C. However, below-freezing weather starting on day 23 through the end of the experiment lowered ambient temperature in the laboratory, causing water temperatures to drop as low as 17.3 °C.

4.4 Influent and Effluent pH

BSF influent and effluent pH values were recorded throughout the operation of the BSFs and are shown in Fig. 4.2

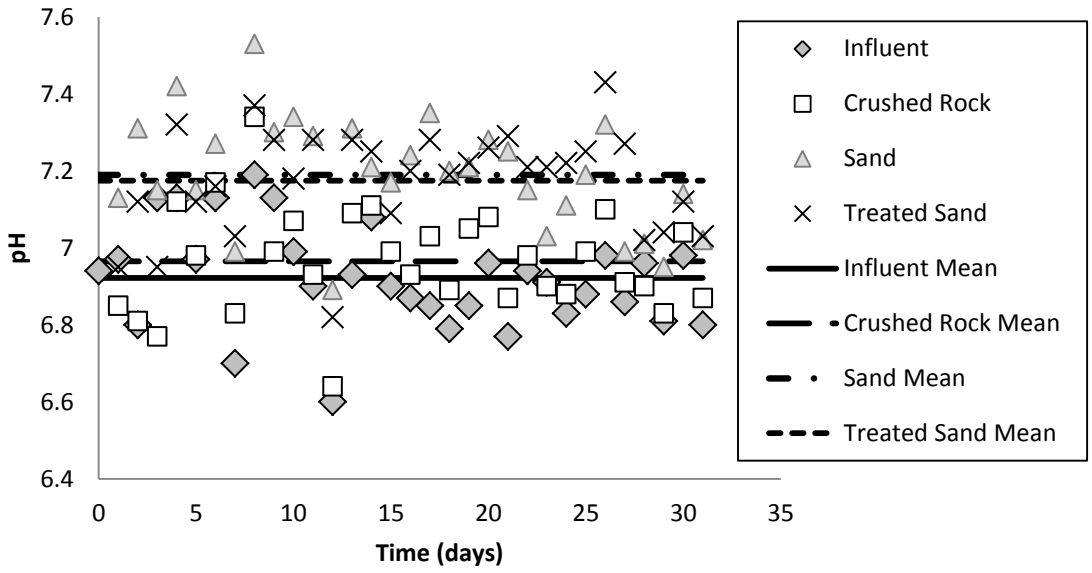

Figure 4.2: Biosand filter influent and effluent pH.

The preceding figure shows that pH remained between 6.6 and 7.6 for all influent and effluent streams during the experiment. The mean effluent pH for sand and treated sand BSFs are significantly higher than both the mean effluent pH for the crushed rock BSF and the mean influent pH ($P < 0.001$ for all four cases).

4.5 Influent and Effluent Conductivity

BSF influent and effluent conductivity values were recorded throughout operation of the BSFs and are shown in Fig. 4.3. From the beginning of the experiment through day 2, conductivity values were omitted due to a failing conductivity probe.

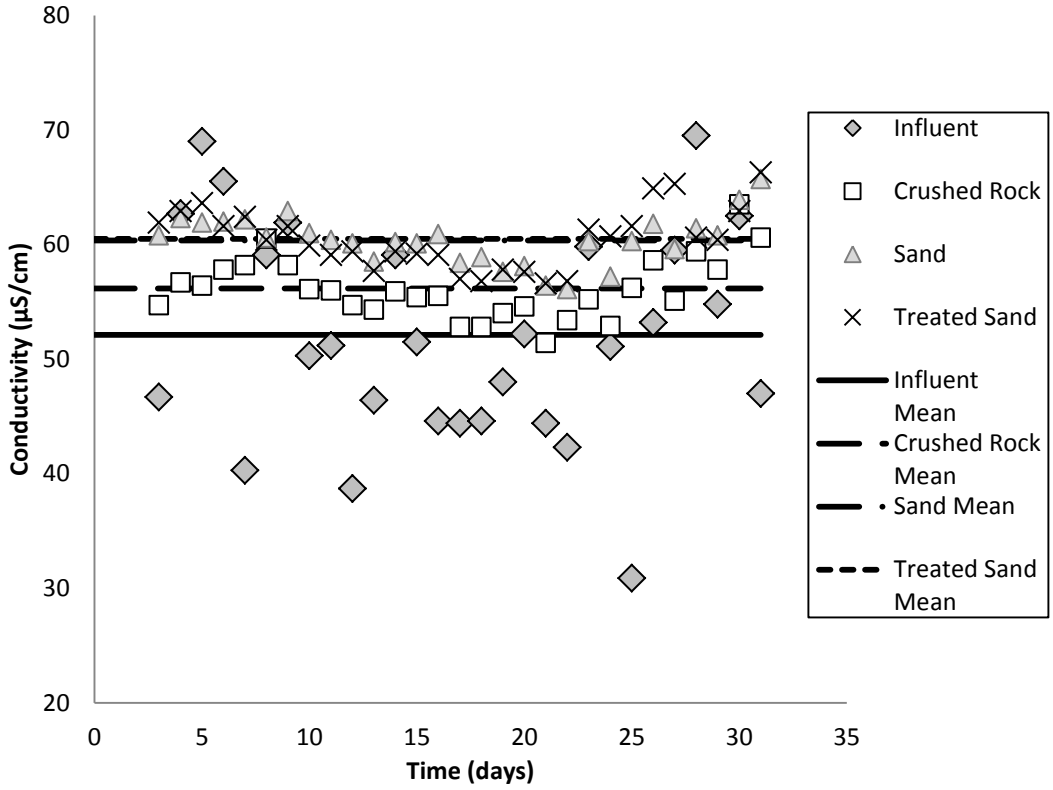

Figure 4.3: Biosand filter influent and effluent conductivity.

The mean conductivity of the filter influent was significantly lower than the mean conductivities of each filter effluent (see Table 4-3 for P-values). However, the mean effluent conductivities of the sand and treated sand filters were significantly higher than the crushed rock filter (see Table 4-3 for P-values). Table 4-3 summarizes the mean influent and effluent conductivities and P-values of significantly different mean conductivities.

Table 4-3: Mean conductivity values and P-values for significantly different mean conductivities.

	Influent	Crushed Rock Effluent	Sand Effluent	Treated Sand Effluent
Mean Conductivity (µS/cm)	52.1	56.2	60.4	60.5
Influent		P<0.05	P<0.001	P<0.001
Crushed Rock Effluent			P<0.001	P<0.001

4.6 Influent and Effluent Turbidity

Influent and effluent turbidity was recorded throughout the operation of the BSFs and are shown in Fig. 4.4.

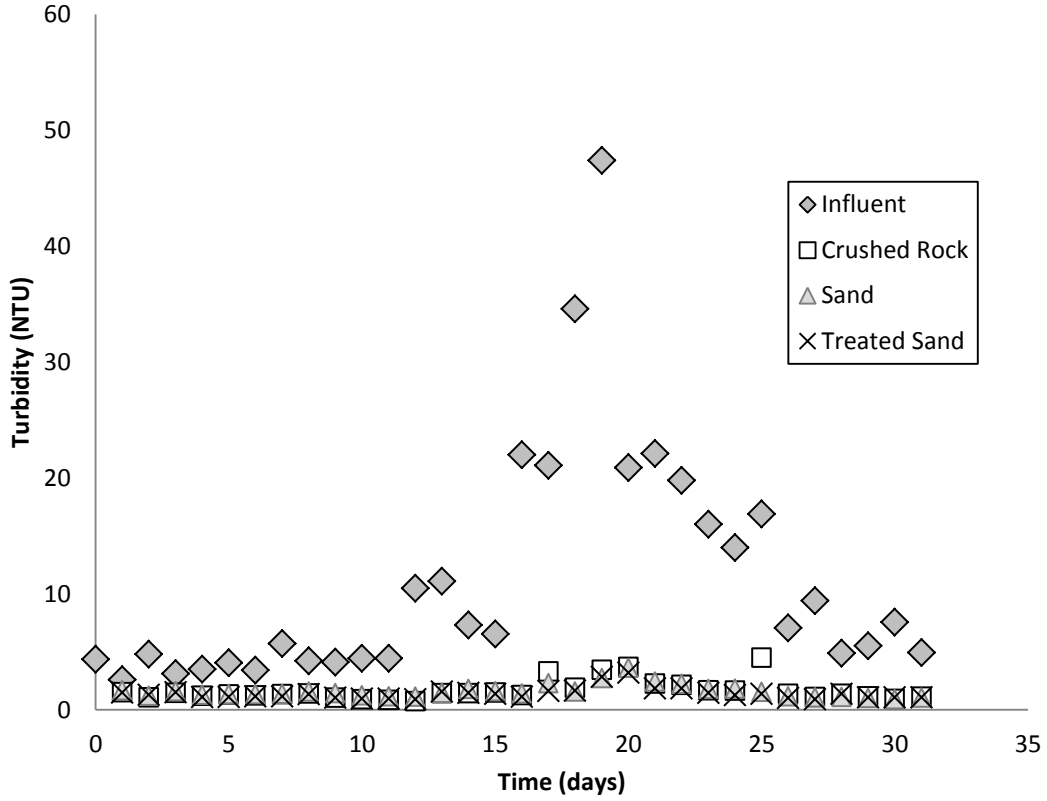

Figure 4.4: Biosand filter influent and effluent turbidity.

Mean influent turbidity was significantly greater than the mean effluent turbidities from each filter ($P < 0.001$). Significant differences were not observed between mean effluent turbidities of the three filters. Influent turbidity remained below 6 NTU from the beginning of the experiment to day 12, where influent turbidity began to climb until day 19 to a peak above 47 NTU. This turbidity event correlates with discharge data for the Willamette River, as is shown in Fig. 4.5. River discharge data was

acquired from the USGS National Water Information System at site number 14174000 located downstream of Corvallis in Albany, Oregon (U.S. Geological Survey 2012).

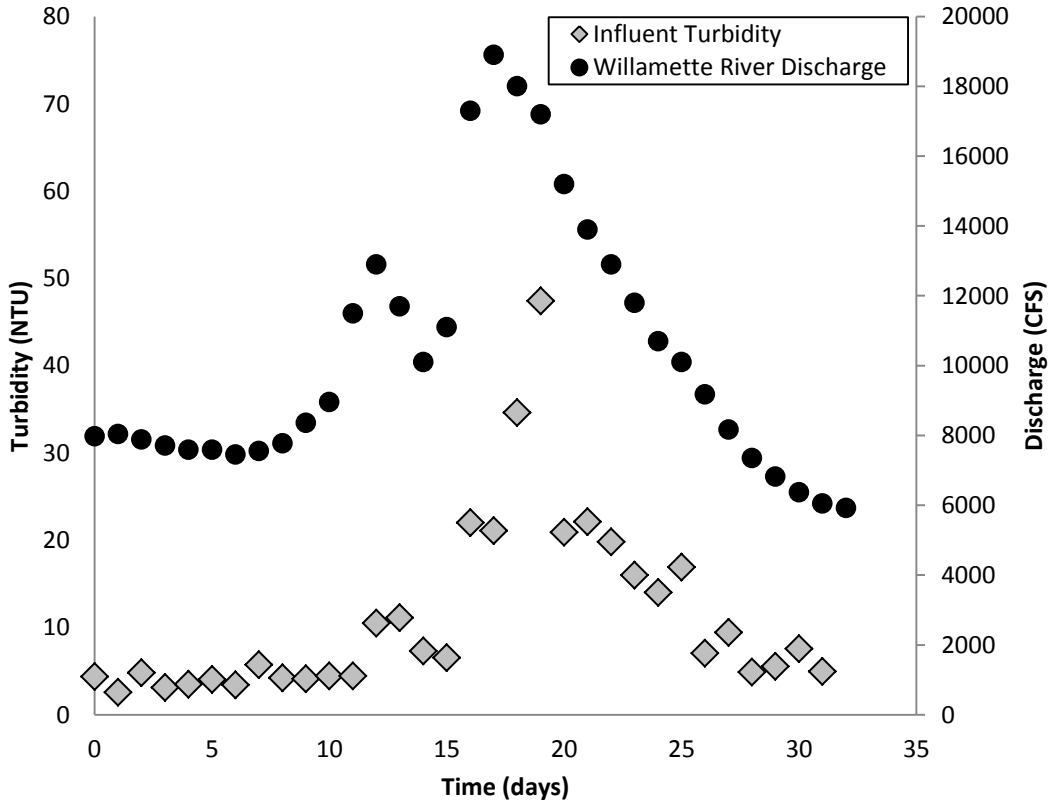

Figure 4.5: Willamette River discharge comparison to influent turbidity. Discharge data acquired from USGS National Water Information System (U.S. Geological Survey 2012).

4.7 Hydraulic Conductivity of Filter Media

After dosing a particular filter, the time was measured for the pressure head to drop from an initial to a final known value. By applying the falling head permeability test described in *Eq. 3.2*, hydraulic conductivity was estimated and is displayed in Fig. 4.6.

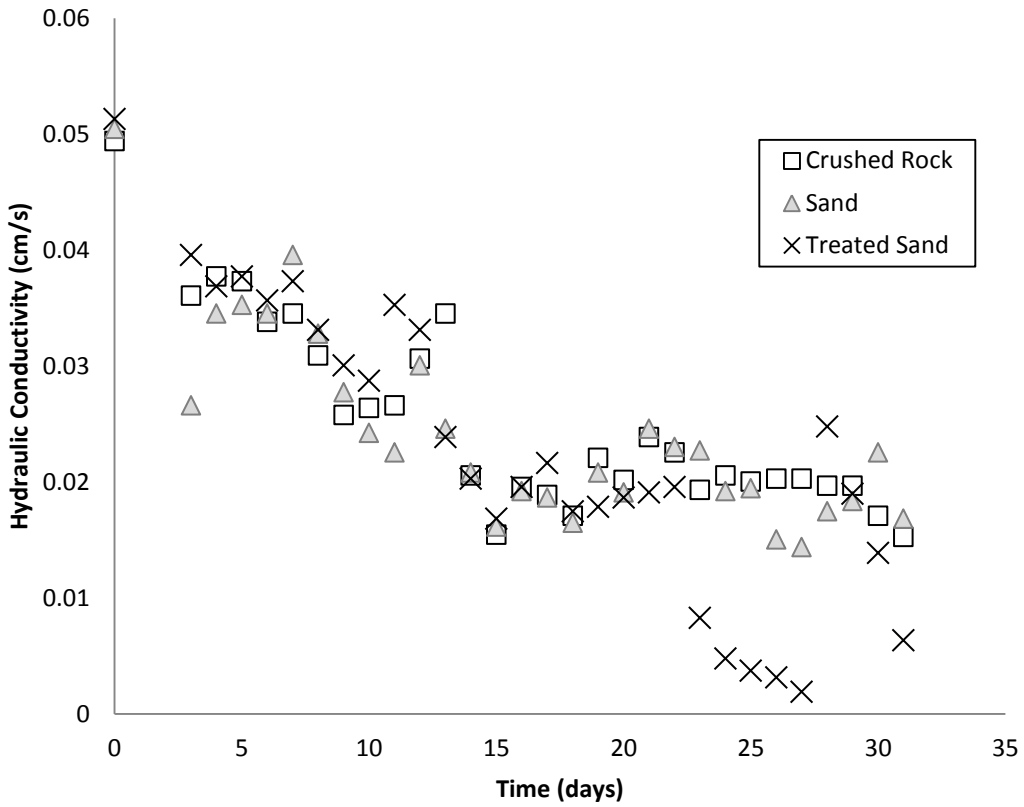

Figure 4.6: Estimated hydraulic conductivity of filter bed media.

Significant differences in overall mean hydraulic conductivity for the crushed rock, beach sand, and heat-treated beach sand were not observed. However, the heat treated beach sand media filter did take a significantly longer time to filter the specified 300 mL of influent than both the crushed rock media filter ($P < 0.05$) and the beach sand media filter ($P < 0.05$). Refer to *Appendix J* for hydraulic conductivity and filtration time data.

Between days 23 and 27, hydraulic conductivity through the heat-treated beach sand filter quickly decreased to the point of inoperability. After sample collection from the heat-treated beach sand filter on day 27, the surface of the filter bed was cleaned as described by standard BSF operation procedures (Manz 2009) such that the hydraulic conductivity was raised to operable levels.

4.8 Influent and Effluent Dissolved Oxygen

Influent and effluent dissolved oxygen (DO) concentration was monitored daily throughout the duration of the experiment and is summarized in Fig. 4.7.

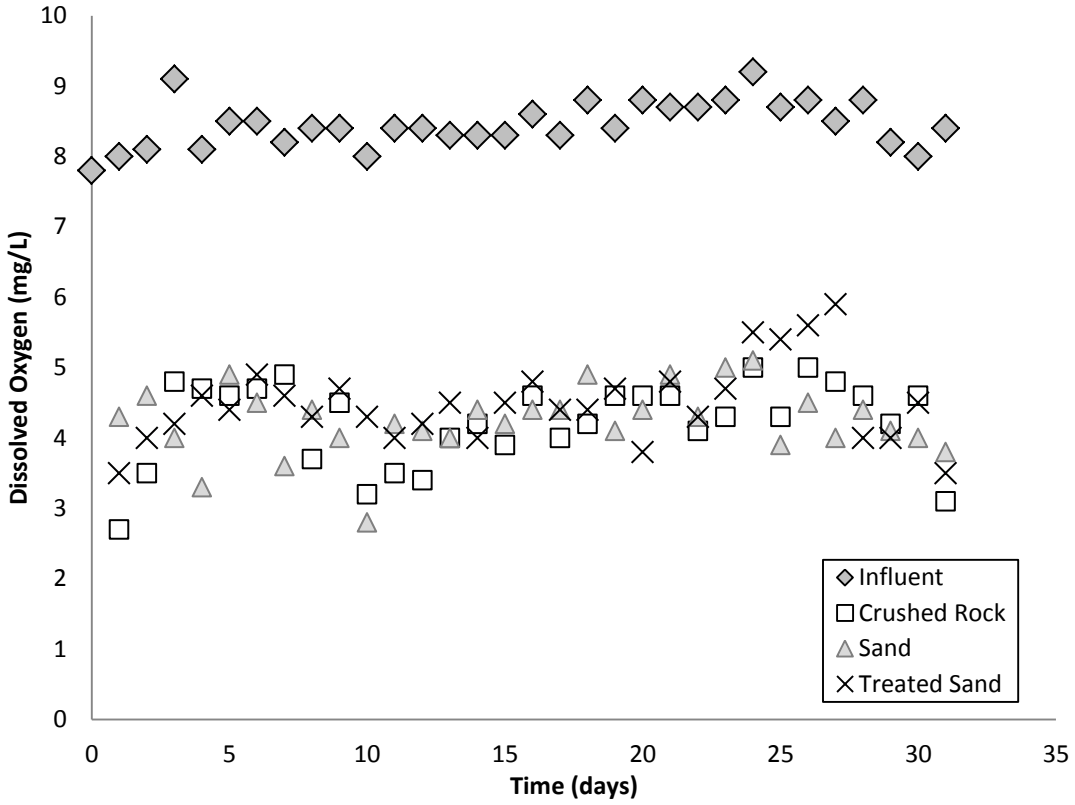

Figure 4.7: Biosand filter influent and effluent dissolved oxygen concentrations.

Mean influent DO concentration was significantly higher than each filter's mean effluent concentrations ($P < 0.001$), while significant differences between the mean DO of filter effluents were not observed. The highest four instances of effluent DO all occurred in the heat-treated beach sand filter within the last four days before the filter surface was cleaned (days 24 to 27). These four points coincide with the decrease of hydraulic conductivity seen in Fig. 4.6 and also occurred during the decrease of influent and effluent temperatures displayed in Fig. 4.1.

4.9 Influent and Effluent E. coli Concentration

From day 0 to 13 of filter operation, target influent concentration was 300 CFU/mL.

Figure 4.8 shows all influent and effluent *E. coli* CFU concentrations for days 0 to 13.

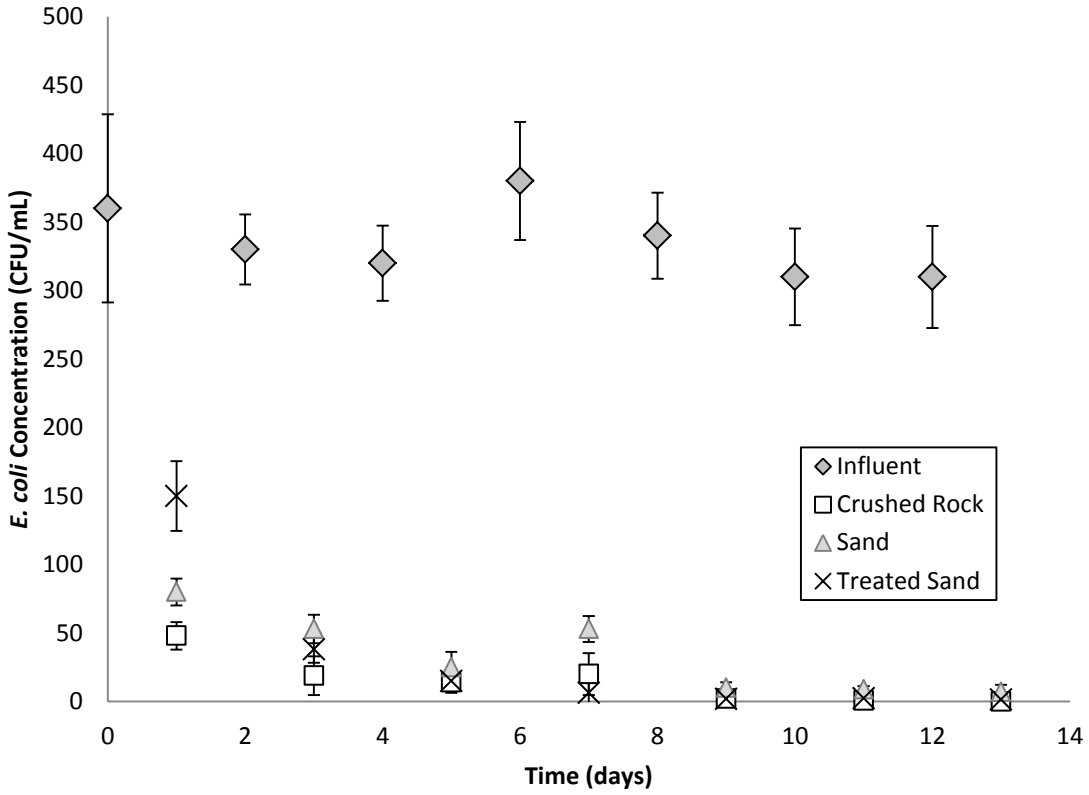

Figure 4.8: Influent and effluent concentrations of *E. coli* during 300 CFU/mL target influent dosing. Error bars represent 95% confidence intervals.

Influent *E. coli* concentrations ranged from 310 to 380 CFU/mL while initial effluent concentrations ranged from 48 CFU/mL (crushed rock media filter) to 150 CFU/mL (heat-treated beach sand filter). For better comparison of effluent *E. coli* concentrations, Fig. 4.9 focuses only on the effluent results for days 0 through 13.

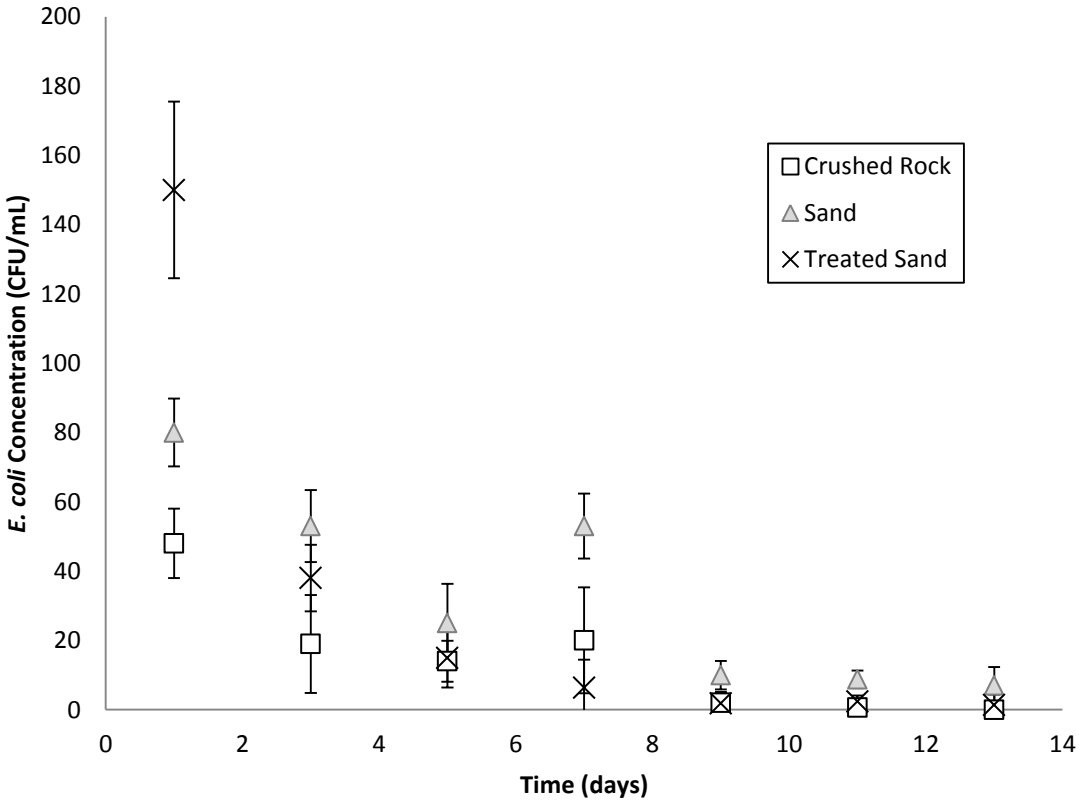

Figure 4.9: Effluent concentrations of *E. coli* during 300 CFU/mL target influent dosing. Error bars represent 95% confidence intervals.

Figure 4.10 plots influent and effluent *E. coli* concentrations for days 14 through 31, where influent *E. coli* target concentration was 3000 CFU/mL. Target influent concentration was increased to prevent effluent *E. coli* plate counts of less than 1 CFU/mL due to high *E. coli* removal efficiencies. Influent concentration ranged from 2300 to 3500 CFU/mL during this period.

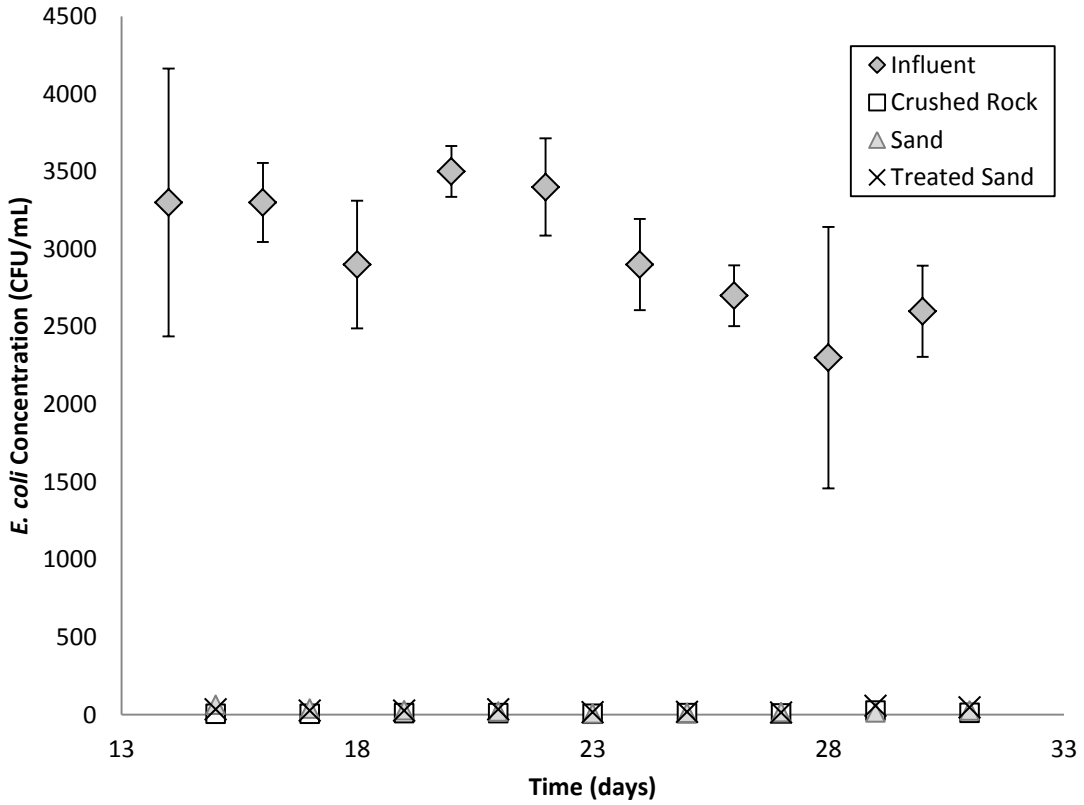

Figure 4.10: Influent and effluent concentrations of *E. coli* during 3000 CFU/mL target influent dosing. Error bars represent 95% confidence intervals.

For better comparison of effluent *E. coli* concentrations, Fig. 4.11 focuses only on the effluent results for days 14 through 31.

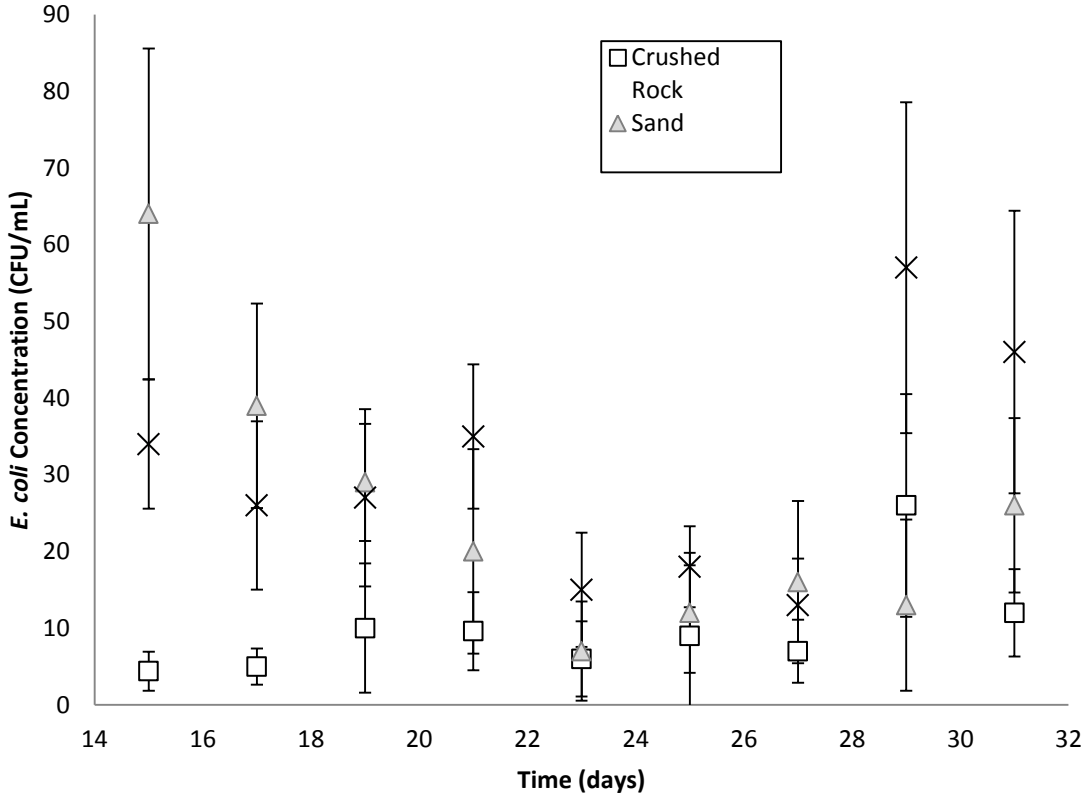

Figure 4.11: Effluent concentrations of *E. coli* during 3000 CFU/mL target influent dosing. Error bars represent 95% confidence intervals.

After the transition from 300 CFU/mL to 3000 CFU/mL of *E. coli* influent, both beach sand and heat-treated beach sand filters gradually approached the sub-20 CFU/mL effluent concentrations achieved by the crushed rock filter on the first effluent

sampling after the transition. The two highest effluent *E. coli* concentrations for the heat-treated beach sand filter occurred on the last two days of sampling. This jump in effluent *E. coli* concentration follows the cleaning of the heat-treated beach sand filter, which occurred between samples taken on days 27 and 29. Table 4-4 summarizes significant differences between *E. coli* concentrations of each filter effluent.

Table 4-4: Comparison of each filter effluent for significant differences in daily *E. coli* concentrations.

Day	Crushed Rock Filter vs. Beach Sand Filter		Crushed Rock Filter vs. Heat-Treated Beach Sand Filter		Beach Sand Filter vs. Heat-Treated Beach Sand Filter	
	Significantly lower effluent E. coli concentration	***P-Value***	***Significantly lower effluent E. coli concentration***	***P-Value***	***Significantly lower effluent E. coli concentration***	***P-Value***
1	Crushed Rock	< 0.001	Crushed Rock	< 0.001	Beach Sand	< 0.001
3	Crushed Rock	< 0.001	Crushed Rock	< 0.01	Heat-Treated Beach Sand	< 0.01
5	Crushed Rock	< 0.01	no significant difference	> 0.1	Heat-Treated Beach Sand	< 0.05
7	Crushed Rock	< 0.001	Heat-Treated Beach Sand	< 0.05	Heat-Treated Beach Sand	< 0.001
9	Crushed Rock	< 0.001	no significant difference	> 0.1	Heat-Treated Beach Sand	< 0.001
11	Crushed Rock	< 0.001	Crushed Rock	< 0.01	Heat-Treated Beach Sand	< 0.001
13	Crushed Rock	< 0.01	no significant difference	> 0.1	Heat-Treated Beach Sand	< 0.01
15	Crushed Rock	< 0.001	Crushed Rock	< 0.001	Heat-Treated Beach Sand	< 0.01
17	Crushed Rock	< 0.001	Crushed Rock	< 0.001	Heat-Treated Beach Sand	< 0.05
19	Crushed Rock	< 0.001	Crushed Rock	< 0.01	no significant difference	> 0.1
21	Crushed Rock	< 0.05	Crushed Rock	< 0.001	Beach Sand	< 0.01
23	no significant difference	> 0.1	Crushed Rock	< 0.01	Beach Sand	< 0.01
25	no significant difference	> 0.1	Crushed Rock	< 0.01	Beach Sand	< 0.05
27	Crushed Rock	< 0.05	Crushed Rock	< 0.01	no significant difference	> 0.1
29	Beach Sand	< 0.05	Crushed Rock	< 0.01	Beach Sand	< 0.001
31	Crushed Rock	< 0.01	Crushed Rock	< 0.001	Beach Sand	< 0.01

Effluent from the crushed rock filter was significantly lower in *E. coli* concentration than the effluent from the beach sand filter for approximately 80% of all days recorded with the only exceptions occurring on days 23, 25, and 29. Similarly, effluent from the crushed rock filter had significantly lower *E. coli* concentrations than the effluent from the heat-treated beach sand filter for approximately 80% of all days recorded with exceptions occurring on days 5, 9, 7 and 13.

When the beach sand filter and the heat-treated beach sand filter are compared, 50% of all recorded days showed the heat-treated beach sand filter with a significantly lower effluent *E. coli* concentration, while 40% of all recorded days showed the beach sand filter producing significantly lower *E. coli* effluent concentration. This suggests that the heat-treated beach sand and the untreated beach sand used in this experiment may not differ enough to notably influence *E. coli* removal.

Additionally, the mean of all plate counts of each filter effluent was used to determine mean *E. coli* effluent concentration for each filter. Table 4-5 shows these mean values along with P-values representing significant differences between these means.

Table 4-5: Mean effluent *E. coli* concentration values and P-values for filters with significantly different mean concentrations.

	Crushed Rock Filter	***Beach Sand Filter***	***Heat-Treated Beach Sand Filter***
Mean effluent E. coli concentration (CFU/mL)	12	29	30
Crushed Rock Filter		$P < 0.001$	$P < 0.001$

Table 4-5 shows that the mean effluent *E. coli* concentrations for the beach sand and the heat treated beach sand filters were over twice the mean effluent concentration of the crushed rock filter. Beach sand filter and heat-treated beach sand filter mean effluent *E. coli* concentrations were not statistically different ($P > 0.1$), suggesting that the beach sand and the heat-treated beach sand used in this experiment did not differ enough to notably influence *E. coli* removal.

4.10 Removal Efficiency of E. coli

Removal efficiency associated with a particular effluent sample was calculated with the nearest preceding influent sample *E. coli* concentration such that the influent and effluent samples were both representative of the same pore volume. Figure 4.12 shows removal efficiencies during the experiment with 95% confidence intervals determined through propagation of error. In order to determine removal efficiency, both influent and effluent plate counts must be used in the calculation, thus error from both influent and effluent plate count error must be considered to determine the error of the calculated removal efficiency.

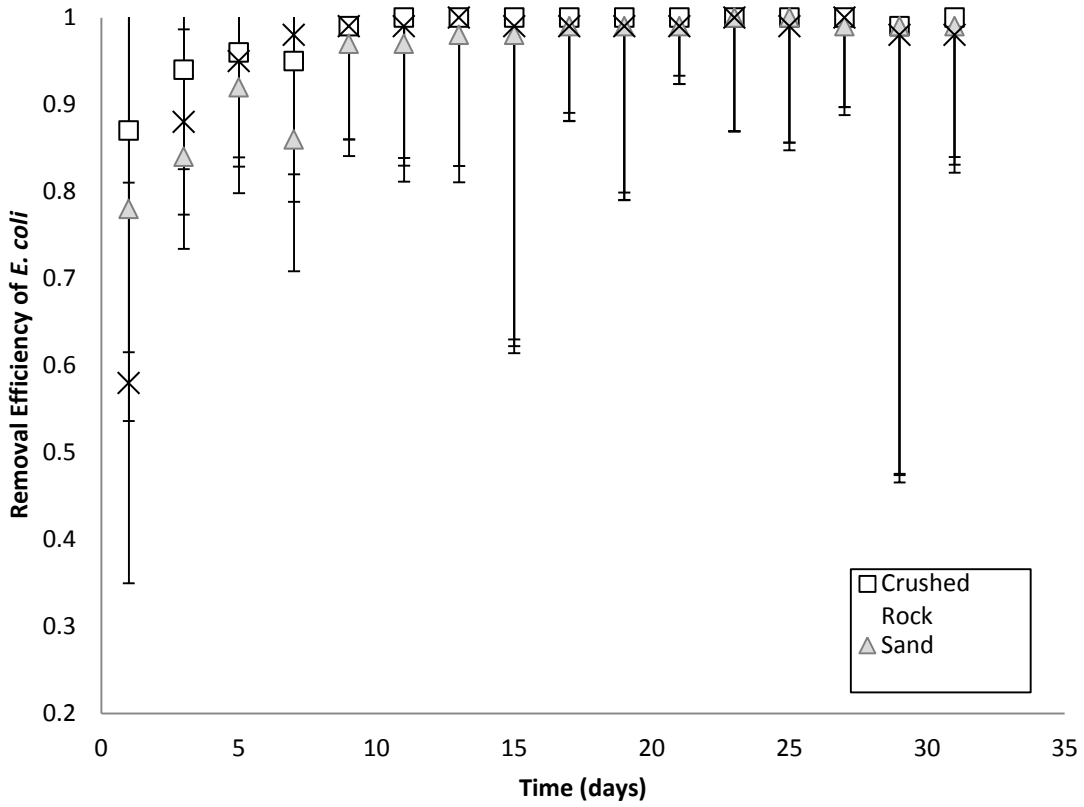

Figure 4.12: Biosand filter removal efficiencies of *E. coli*. Error bars represent 95% confidence intervals.

95% confidence intervals were relatively large when compared to the distribution of data points on a particular day, preventing the identification of significant differences in removal efficiencies between the three filters. Only four significant differences in *E. coli* removal efficiencies were observed between the three filters; all of which occurred during the first week of operation. A summary of these significant differences and their associated P-values are displayed in Table 4-6.

Table 4-6: Summary of significant differences in *E. coli* removal efficiency and respective P-values.

Day of Observation	*E. coli removal efficiency is significantly greater in the:*	*P-Value*
1	crushed rock filter than the heat-treated beach sand filter	< 0.01
1	beach sand filter than the heat-treated beach sand filter	< 0.05
3	crushed rock filter than the beach sand filter	< 0.05
7	heat-treated beach sand filter than the beach sand filter	< 0.05

4.11 Effects of Residence Time on E. coli Removal

On day 24, effluent samples were collected as the final 1 L of influent water was passing through each filter. These samples have a small residence time (t_R) within the filter when compared to the typical 20-24 hour residence time of samples shown in Section 4.9. Table 4-7 summarizes the collected data and notes significant differences in *E. coli* concentration and removal efficiencies with varying residence time.

Table 4-7: *E. coli* concentration and removal efficiencies with varying residence time. P-values show if effluent *E. coli* concentrations and removal efficiencies significantly differ between samples collected at short (t_R < 4 hr) and long ($t_R \approx$ 24 hr) residence times.

Filter	*Influent E. Coli Concentration (CFU/mL)*	**Short Residence Time (t_R < 4 hr)**		**Long Residence Time ($t_R \approx$ 24 hr)**		*Does effluent concentration significantly differ with change in t_R?*	*P-Value*	*Does removal efficiency significantly differ with change in t_R?*	*P-Value*
		Effluent E. coli Concentration (CFU/mL)	*E. coli Removal Efficiency*	*Effluent E. coli Concentration (CFU/mL)*	*E. coli Removal Efficiency*				
Crushed Rock	2900	640	0.78	9	> 0.99	Yes	< 0.001	Yes	< 0.01
Beach Sand	2900	280	0.90	12	> 0.99	Yes	< 0.001	Yes at 90% Confidence	< 0.1
Heat-Treated Beach Sand	2900	380	0.87	18	0.99	Yes	< 0.001	Yes	< 0.05

P-values listed in Table 4-7 show that short residence time samples had significantly higher effluent *E. coli* concentrations and significantly lower *E. coli* removal efficiencies than long residence time samples. Table 4-8 shows the statistical comparison of short residence time effluent *E. coli* concentrations, indicating that beach sand and heat-treated beach sand effluents were significantly lower than crushed rock effluent. Beach sand effluent was also significantly lower than heat-treated beach sand effluent in *E. coli* concentration.

Table 4-8: Summary of significant differences in effluent *E. coli* concentrations of each filter for short residence time samples.

	Influent	***Crushed Rock Effluent***	***Beach Sand Effluent***	***Heat-Treated Beach Sand Effluent***
E. coli Concentration (CFU/mL)	2900	640	280	380
Crushed Rock			P < 0.001	P < 0.001
Beach Sand				P < 0.01

Table 4-8 indicates that beach sand and heat-treated beach sand effluents were significantly lower than crushed rock effluent. Beach sand effluent was also significantly lower than heat-treated beach sand effluent in *E. coli* concentration.

Table 4-9 summarizes *E. coli* removal efficiencies for short residence time samples and highlights significant differences between each filter's removal efficiencies.

Table 4-9: Summary of significant differences in *E. coli* removal efficiencies between each filter for short residence time samples.

	Crushed Rock Filter	***Beach Sand Filter***	***Heat-Treated Beach Sand Filter***
Removal Efficiency	0.78	0.90	0.87
Crushed Rock		$P < 0.05$	$P < 0.1$

Table 4-9 shows the removal efficiency achieved by the crushed rock filter was significantly lower than both beach sand and heat-treated beach sand filters with short residence time sampling.

4.12 Selection of Media

Despite effective removal of *E. coli* with BSFs containing beach sand and heat-treated beach sand media, crushed rock should still be the preferred media for BSFs when available due to lower *E. coli* concentrations observed in the crushed rock BSF effluent. In situations where crushed rock is not available, beach sand with appropriate hydraulic conductivity, minimal organic carbon content, and minimal potential for contamination should be sourced. Prior to use of a beach sand media BSF for drinking water, it is imperative that the installation be followed by a series of effluent monitoring events for *E. coli* or fecal coliforms in the case that the source sand is biologically contaminated. This could be avoided by heat-treating the filter media in a kiln, but this could prove to be prohibitive in situations where fuel is difficult or expensive to obtain. It is also important to recognize that beach sand and heat-treated beach sand BSFs with crushed rock BSFs have not been assessed for the removal efficiency of viruses, so conclusions of pathogen removal in this research is limited to the scope of pathogenic Gram-negative bacteria.

5 Discussion

Biosand filters with crushed rock, beach sand, and heat-treated beach sand media all achieved stable removal efficiencies of 99% or greater after filter maturation and prior to filter surface cleaning, indicating that it is possible to effectively use beach sand and heat-treated beach sand in Biosand filters for the removal of bacteria. Although *E. coli* removal efficiencies did not significantly differ between filters, crushed rock filter effluent was significantly lower in *E. coli* concentration than both the beach sand filter and heat-treated beach sand filter on 80% of recorded days. Mean effluent concentrations of *E. coli* from the crushed rock filter were less than half of the mean effluent concentrations from the beach sand and heat-treated beach sand filters. This could be attributed to differences in chemical or physical surface properties of each filter media, which can affect the probability of particle collision and particle/biofilm attachment to the media surface due to variations in media surface area, hydrodynamic/spatial conditions, and differences in media particle charge. Previous research has found that surfaces with higher irregularity promotes biofilm attachment (Chang and Rittmann 1988; Fox, Suidan, and Bandy 1990). Because beach sand particles are typically more weathered than crushed rock, the surface of crushed rock may better accommodate biofilm and particle attachment due to greater surface

irregularity, greater surface area, and less potential for media shifting, which could dislodge existing biofilm.

Mean effluent conductivities of each filter was significantly higher than the mean conductivity of the influent. This indicates that all three filters are increasing the ion concentration of water passing through the filters. Additionally, the mean effluent conductivities of the beach sand and heat-treated beach sand filters were significantly higher than the crushed rock filter. This may be due to differences in media mineral composition or residual oceanic salts in the beach sand and heat-treated beach sand media. If both filter media and colloids are negatively charged, increased ionic strength would suggest greater attachment of negatively charged colloids to the filter media. Although differences in mean effluent conductivity between the crushed rock filter and both beach sand filters are statistically significant, the magnitudes of the differences are less than 10 µS/cm, suggesting that differences in *E. coli* removal between the crushed rock, beach sand, and heat treated beach sand were unlikely due to variations of ionic strength.

Decreasing water residence time in the filter significantly increased all three filter effluent *E. coli* concentrations and decreased removal efficiencies, which has been observed in previous research of crushed rock BSFs (Buzunis 1995; Elliott et al.

2008). Two system volumes (4 L total) of influent water were added to each filter. While the second system volume of influent water was being dosed, a 500 mL sample of effluent was collected that had rested in the filter for less than 4 hours and were plated for *E. coli* enumeration. The following day, another pore volume of influent water was added to the filter to collect 500 mL of effluent that had remained in the filter for over 20 hours. The samples were enumerated for *E. coli* concentrations and results were compared to the effluent *E. coli* concentrations in samples collected the previous day. Significant differences between effluent *E. coli* concentrations occurred during short residence time samples, suggesting that the effects of residence time may differ between filter media types. Data collected from the single-day test indicated that the crushed rock filter had the lowest removal efficiency at 0.78, while removal efficiencies in beach sand and heat-treated beach sand filters were significantly higher at 0.90 and 0.87 respectively. These results are surprising considering the mean crushed rock filter effluent was significantly lower than other filter effluents during 24 hour residence time samples. However, more samples are needed to determine if the observed difference between filter effluents is reproducible.

Further characterization of residence time effects on *E. coli* removal could be done by dosing matured filters with 2 pore volumes of seeded water, then collecting and plating samples after 1.5 pore volumes have passed in a given filter. This first sample

point would represent effluent with a minimum residence time in the filter. After the filters reach rest, samples representing longer residence times could be collected by waiting a known duration of time, then dosing a fraction of the pore volume to push another sample through the effluent of each filter. This process could be conservatively repeated for approximately half of a pore volume in order to avoid collecting water with a lower residence time. It would also be beneficial to purge the effluent pipe prior to sampling on each dosing by waiting for an appropriate volume to pass through the effluent. Repeating this sampling process daily for over a week on matured filters could provide useful information for the comparison of *E. coli* removal in crushed rock, beach sand, and heat-treated beach sand filters at different residence times. The results could also be useful for determining optimal filter bed volume with a specific media for a particular water demand.

6 Conclusion

Implementation of Biosand filters can present difficult challenges in locations or situations where material resources are severely limited. When Biosand filters cannot be constructed with crushed rock, a suitable alternative filter media is needed. A month-long experiment with simultaneously operated Biosand filters with crushed rock, beach sand, and heat-treated beach sand media all achieved stable *E. coli* removal efficiencies of 99% or greater. The observed results suggest that it is possible to effectively use beach sand and heat-treated beach sand in Biosand filters for the removal of pathogenic bacteria. However, beach sand and heat-treated beach sand media should only be used as secondary options to crushed rock media due to statistically higher mean effluent *E. coli* concentrations from Biosand filters constructed with beach sand and heat-treated beach sand.

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APPENDICES

Appendix A: Procedure for E.coli Seeding

Previously frozen *E. coli* K-12 culture, supplied by Gabriel C. Iltis, was inoculated into 200 mL of 30 g/L tryptic soy broth (TSB) and incubated at 37°C on a shaker table at 200 rpm for 12 hours. 0.5 mL of autoclaved glycerol as well as 0.5 mL of cultured K-12 *E. coli* was pipetted to sterile 2 mL cyro-vials using aseptic technique. Closed vials were mixed by hand and then stored at -80°C.

100 mL of 8 g/L TSB culture media was autoclaved in 125 mL Erlenmyer flasks for 20 minutes. The autoclaved TSB media was inoculated with 1 mL of frozen *E. coli* K-12. The inoculated TSB was grown to log phase on an incubated shaker table at 35°C for 4 hours. The culture was cooled to 4°C. Filter dosing water with *E. coli* concentrations of approximately 300 cfu/mL and 3000 cfu/mL was prepared by serial dilutions of K-12 culture into Willamette River water collected at H.D. Taylor Water Treatment Plant. *E. coli* concentration was enumerated by the direct plating of 1 mL of sample onto 3M Petrifilm *E. coli*/Coliform plates as described in the manufacturer instructions.

Appendix B: Procedure for the Serial Dilution of Bacteria

Serial dilution of a cell culture is necessary for the enumeration of colony forming units per volume of sample when the cell concentration of a sample results in a culture plate that is too densely populated to count. Successive dilutions of 1:10 were carried out in autoclaved vials using sterile 10 mL and 1 mL pipettes until the desired dilution was obtained. Each successive dilution contained 9 mL of Willamette River water (collected at H.D. Taylor Water Treatment Plant, Corvallis, Oregon) and 1mL of the initial culture or preceding mixed dilution.

Appendix C: Procedure for 3M Petrifilm Plating

Unopened Petrifilm *E. coli*/Coliform Count Plates were stored at 4°C. Opened packages were sealed and stored below 25°C. Prior to handling Petrifilm plates, latex gloves were worn and sterilized with 70% ethanol. The Petrifilm plates were placed on a sterilized, level surface within the sterile hood. Sterilized pipettes were used to transfer 1mL samples onto each Petrifilm plate. The top film of Petrifilm plates were carefully rolled down to avoid entrapment of air. The circular spreader plate was gently pressed against the face of the Petrifilm plate to evenly distribute the inoculum. After letting the plates sit for one minute to allow for the solidification of the Petrifilm gel, plates were moved to an incubator at 35°C for 24 hours. Plates were then removed from the incubator and immediately counted. Blue coloration in a colony indicates the presence of *E. coli* while bubbles surrounding colonies indicate coliform colonies. Refer to manufacturer instructions for additional information.

Appendix D: Procedure for pH/Conductivity Analysis

Conductivity and pH were measured with a VWR SR 60IC meter, VWR SympHony two-cell epoxy platinum conductivity probe (14002-802), and VWR SympHony Waterproof Gel 3-in-1 pH electrode (14002-778). The conductivity and pH meter was calibrated daily with standard solutions, as described by manufacturer instructions. Samples were gently mixed with a magnetic stirrer while calibrating or sampling. Probes were carefully lowered into the sample, being sure to prevent any contact of the stir-bar and the probe. Conductivity or pH readings were allowed to stabilize before recording. Probes were cleaned with deionized water between samples and carefully dabbed dry with a lint-free tissue. Refer to manufacturer product manuals for further information.

Appendix E: Procedure for Dissolved Oxygen and Temperature Analysis

Dissolved oxygen (DO) and temperature were measured with a Hach sensION156, which was calibrated daily with the saturated air method according to manufacturer protocol. Samples were mixed with a magnetic stirrer during DO measurements, as directed by manufacturer protocol. Temperature was recorded after DO was measured to minimize sample exposure to atmospheric oxygen. The DO probe was cleaned with deionized water between samples. For further information, refer to the Hach sensION156 portable multiparameter meter manual.

Appendix F: Procedure for Chemical Oxygen Demand Analysis

Chemical oxygen demand (COD) was assessed with a Hach DRB200 reactor/spectrophotometer and low-range (3-150 mg) COD reagent vials. Samples were homogenized for 30 seconds in a blender. The digestion reactor was heated to 150°C. One blank was prepared by holding a reagent vial at a 45 degree angle while adding 2 mL of deionized water to the vial with a clean volumetric pipette. All samples were prepared in the same manner, except with homogenized sample in place of the deionized water.

Calibration standards were prepared for calibration of the Hach DRB200 reactor/spectrophotometer. Standards of 5, 15, 25, 35, and 40 mg/L COD were prepared by diluting 500 mg/L COD stock solution by respectively adding 1.0, 3.0, 5.0, 7.0, and 8.0 mL of the stock solution to 100 mL volumetric flasks. The contents of each volumetric flask were diluted with deionized water to 100 mL. Flask openings were plugged with stoppers and mixed by inverting each flask 10 times.

The vials were heated in the digestion reactor for two hours. Vials were allowed to cool to below 120°C for 20 minutes. Each vial was inverted several times and placed

into a rack to allow cooling to room temperature. The outside of the vials were cleaned with a damp towel and were then dried.

The five calibration standards and their known concentrations of COD were used for a custom calibration of the colorimeter and the blank was used to zero the device. After the device was calibrated, each sample was inserted into the colorimeter, cover closed, and COD reading was recorded. For more information, refer to Hach Method 8000.

Appendix G: Procedure for Turbidity Analysis

For the analysis of turbidity, a Hach 2100P portable turbidimeter was used. Standards provided with the Hach 2100P were used to conduct a four-point calibration. Standards were mixed prior to calibration by inverting the standard vials several times. Standards were inserted into the turbidimeter and the cover was closed. Each point was calibrated with the appropriate NTU listed on each standard vial.

From 100 mL samples homogenized with a magnetic stirrer, the provided optical vial was filled. The vial was capped and mixed by inverting several times. The vial was inserted into the turbidimeter, cover closed, and the sample turbidity was recorded. Between samples the optical vial was rinsed and dabbed dry with a lint-free paper towel. For further information, refer to the Hach Portable Turbidimeter Model 2100P instrument and procedure manual.

Appendix H: Procedure for Total Organic Carbon Analysis

Total organic carbon (TOC) of the crushed rock media and beach sand media was determined by loss on ignition (LOI). Three samples of approximately 100 g of each raw media (crushed rock media and beach sand media) were collected after media was sorted through a No. 8 sieve and washed according to standard BSF construction procedures (Manz 2010). Crucibles were combusted at 550°C until change in mass was less than 0.5%. Samples were added to the crucibles and dried at 105°C until the change in mass was less than 0.5%. Samples were then combusted at 550°C in a Thermolyne F-A1730 muffle furnace until less than 0.5% change in mass was observed. After being dried or combusted, samples were transferred to desiccators with tongs and allowed to cool until they could be safely weighed.

Appendix I: Procedure for Hydraulic Conductivity Estimation

Each filter was timed for the passing of the first 300 mL after the initial 100 mL purge volume to obtain a hydraulic conductivity estimate for each filter bed by the falling head permeability test described in *Eq. 3.2* on page 36. With this equation, hydraulic conductivity was estimated by timing the filter to pass the first 300 mL (after a 100 mL purge volume) from a 1 L filter dosing. Thus V_0 and V_1 are 900 mL and 600 mL respectively, while the bed length L is 40 cm for this experiment. Using a bed length of 40 cm assumes that the resistance caused by the underdrain and separation layer is negligible.

Appendix J: Data Collection Table

The following table includes all data collected during the filtration experiment.

Table A-1: Data collection table.

Date	Day	Sample Origin	D.O. (mg/L)	pH	Temp. (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Time for head volume to drop from 900 mL to 600 mL (s)	Falling head based hydraulic conductivity (cm/s)	*E. coli* Plate Counts					Notes:
										1	2	3	4	5	
11/8/11	0	Influent	7.8	6.94	23.0	145.5	4.36			375	370	311	343	396	Hydraulic conductivity determined by Darcy's law by the constant-head method during filter comissioning.
		Out Control							0.049						
		Out Sand							0.050						
		Out Treated							0.051						
11/9/11	1	Influent	8.0	6.97	22.8	53.5	2.58								
		Out Control	2.7	6.85	22.8	76.9	1.51			56	47	46	47	42	
		Out Sand	4.3	7.13	22.7	91.3	1.67			74	78	87	78	83	
		Out Treated	3.5	6.95	22.7	93.9	1.48			158	137	130	136	163	
11/10/11	2	Influent	8.1	6.80	22.8	84.9	4.81			323	344	328	338	314	Faulty conductivity probe.
		Out Control	3.5	6.81	23.2	79.6	1.08								
		Out Sand	4.6	7.31	23.1	92.0	1.22								
		Out Treated	4.0	7.12	23.1	90.0	1.29								
11/11/11	3	Influent	9.1	7.13	22.6	46.7	3.12								Replaced conductivity probe.
		Out Control	4.8	6.77	22.6	54.7	1.46	450	0.036	12	31	20	17	16	
		Out Sand	4.0	7.15	22.6	60.8	1.59	610	0.027	47	58	52	58	48	
		Out Treated	4.2	6.95	22.6	61.9	1.52	410	0.040	40	31	43	34	40	
11/12/11	4	Influent	8.1	7.13	22.9	62.7	3.51			323	321	317	299	343	
		Out Control	4.7	7.12	22.9	56.7	1.20	430	0.038						
		Out Sand	3.3	7.42	22.9	62.3	1.29	470	0.035						
		Out Treated	4.6	7.32	22.9	62.9	1.10	440	0.037						

Table A-1: Data collection table (Continued).

Date	Day	Sample Origin	D.O. (mg/L)	pH	Temp. (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Time for head volume to drop from 900 mL to 600 mL (s)	Falling head based hydraulic conductivity (cm/s)	*E. coli* Plate Counts					Notes:
										1	2	3	4	5	
11/13/11	5	Influent	8.5	6.97	24.0	69.0	4.05								
		Out Control	4.6	6.98	24.0	56.4	1.32	435	0.037	10	14	11	17	16	
		Out Sand	4.9	7.15	24.1	61.9	1.34	460	0.035	23	22	27	19	34	
		Out Treated	4.4	7.12	24.2	63.6	1.09	430	0.038	22	13	12	16	11	
11/14/11	6	Influent	8.5	7.13	23.1	65.5	3.43			392	406	380	345	391	1 coliform found in influent.
		Out Control	4.7	7.17	23.8	57.8	1.25	480	0.034						
		Out Sand	4.5	7.27	23.8	62.0	1.36	470	0.035						
		Out Treated	4.9	7.16	23.8	61.6	1.16	455	0.036						
11/15/11	7	Influent	8.2	6.70	23.4	40.3	5.71								
		Out Control	4.9	6.83	23.8	58.2	1.36	470	0.035	20	12	24	12	30	
		Out Sand	3.6	6.99	23.9	62.2	1.37	410	0.040	52	58	49	58	48	
		Out Treated	4.6	7.03	23.9	62.4	1.16	435	0.037	6	7	13	3	3	
11/16/11	8	Influent	8.4	7.19	22.3	59.1	4.21			334	353	333	360	318	
		Out Control	3.7	7.34	22.7	60.5	1.40	525	0.031						
		Out Sand	4.4	7.53	22.7	60.6	1.58	495	0.033						
		Out Treated	4.3	7.37	22.6	60.4	1.34	490	0.033						
11/17/11	9	Influent	8.4	7.13	23.7	61.9	4.12								
		Out Control	4.5	6.99	23.8	58.2	1.04	630	0.026	0	3	2	1	4	
		Out Sand	4.0	7.30	24.0	62.9	1.44	585	0.028	12	10	11	12	7	
		Out Treated	4.7	7.28	24.0	61.6	1.04	540	0.030	1	3	4	1	0	

Table A-1: Data collection table (Continued).

Date	Day	Sample Origin	D.O. (mg/L)	pH	Temp. (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Time for head volume to drop from 900 mL to 600 mL (s)	Falling head based hydraulic conductivity (cm/s)	*E. coli* Plate Counts					Notes:
										1	2	3	4	5	
11/18/11	10	Influent	8.0	6.99	22.6	50.3	4.44			329	285	312	287	315	
		Out Control	3.2	7.07	23.2	56.1	0.92	615	0.026						
		Out Sand	2.8	7.34	23.3	61.0	1.23	670	0.024						
		Out Treated	4.3	7.18	23.2	59.9	0.96	565	0.029						
11/19/11	11	Influent	8.4	6.90	21.6	51.2	4.43								
		Out Control	3.5	6.93	22.0	56.0	0.84	610	0.027	0	1	1	0	1	
		Out Sand	4.2	7.29	21.9	60.4	1.16	720	0.023	8	9	10	7	10	
		Out Treated	4.0	7.28	21.9	59.1	0.97	460	0.035	3	2	1	3	3	
11/20/11	12	Influent	8.4	6.60	21.4	38.7	10.50			292	341	320	302	306	
		Out Control	3.4	6.64	22.0	54.7	0.74	530	0.031						
		Out Sand	4.1	6.89	22.0	60.1	1.12	540	0.030						
		Out Treated	4.2	6.82	22.0	59.4	0.91	490	0.033						
11/21/11	13	Influent	8.3	6.93	22.7	46.4	11.10								
		Out Control	4.0	7.09	23.0	54.3	1.43	470	0.035	0	0	0	0	0	
		Out Sand	4.0	7.31	23.2	58.5	1.46	660	0.025	9	9	4	4	9	
		Out Treated	4.5	7.28	23.2	57.7	1.58	680	0.024	3	3	0	1	0	
11/22/11	14	Influent	8.3	7.08	22.7	59.1	7.31			307	346	309	293	399	Influent concentration increased x 10. Multiply counts by 10 for actual concentration.
		Out Control	4.2	7.11	23.7	55.9	1.42	790	0.021						
		Out Sand	4.4	7.21	23.6	60.2	1.78	780	0.021						
		Out Treated	4.0	7.25	23.6	59.4	1.45	800	0.020						

Table A-1: Data collection table (Continued).

Date	Day	Sample Origin	D.O. (mg/L)	pH	Temp. (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Time for head volume to drop from 900 mL to 600 mL (s)	Falling head based hydraulic conductivity (cm/s)	*E. coli* Plate Counts					Notes:
										1	2	3	4	5	
11/23/11	15	Influent	8.3	6.90	23.3	51.5	6.54								
		Out Control	3.9	6.99	23.6	55.4	1.49	1050	0.015	3	6	3	5	5	
		Out Sand	4.2	7.17	23.6	60.1	1.61	1005	0.016	52	75	57	60	78	
		Out Treated	4.5	7.09	23.8	59.2	1.39	965	0.017	36	35	26	36	35	
11/24/11	16	Influent	8.6	6.87	22.1	44.6	22.00			336	317	350	317	335	
		Out Control	4.6	6.93	23.6	55.5	1.27	830	0.020						
		Out Sand	4.4	7.24	23.5	60.9	1.40	845	0.019						
		Out Treated	4.8	7.20	23.7	59.1	1.12	830	0.020						
11/25/11	17	Influent	8.3	6.85	22.9	44.4	21.10								
		Out Control	4.0	7.03	23.0	52.8	3.30	860	0.019	5	5	6	6	3	
		Out Sand	4.4	7.35	23.1	58.4	2.30	870	0.019	32	42	44	31	45	
		Out Treated	4.4	7.28	23.2	57.0	1.62	750	0.022	19	31	32	22	27	
11/26/11	18	Influent	8.8	6.79	22.1	44.6	34.60			273	301	291	310	263	
		Out Control	4.2	6.89	23.3	52.8	1.90	950	0.017						
		Out Sand	4.9	7.20	23.4	58.9	1.57	985	0.016						
		Out Treated	4.4	7.19	23.4	56.8	1.66	930	0.017						
11/27/11	19	Influent	8.4	6.85	23.3	48.0	47.40								
		Out Control	4.6	7.05	23.6	54.0	3.46	735	0.022	7	5	11	14	15	
		Out Sand	4.1	7.21	23.6	57.6	2.73	780	0.021	28	27	25	35	31	
		Out Treated	4.7	7.22	23.6	57.8	2.84	910	0.018	21	23	35	31	24	

Table A-1: Data collection table (Continued).

Date	Day	Sample Origin	D.O. (mg/L)	pH	Temp. (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Time for head volume to drop from 900 mL to 600 mL (s)	Falling head based hydraulic conductivity (cm/s)	*E. coli* Plate Counts					Notes:
										1	2	3	4	5	
11/28/11	20	Influent	8.8	6.96	22.5	52.2	20.90			346	351	341	355	337	
		Out Control	4.6	7.08	23.4	54.6	3.69	805	0.020						
		Out Sand	4.4	7.28	23.5	58.1	3.60	850	0.019						
		Out Treated	3.8	7.26	23.6	57.6	3.21	870	0.019						
11/29/11	21	Influent	8.7	6.77	22.2	44.4	22.10								
		Out Control	4.6	6.87	22.8	51.4	2.25	680	0.024	9	6	13	9	11	
		Out Sand	4.9	7.25	23.0	56.4	2.43	660	0.025	20	30	23	15	13	
		Out Treated	4.8	7.29	23.0	56.6	1.85	850	0.019	32	41	38	29	36	
11/30/11	22	Influent	8.7	6.94	22.2	42.3	19.80			318	353	338	360	333	
		Out Control	4.1	6.98	23.4	53.4	2.14	720	0.023						
		Out Sand	4.3	7.15	23.6	56.1	2.24	705	0.023						
		Out Treated	4.3	7.21	23.6	56.8	1.86	830	0.020						
12/1/11	23	Influent	8.8	6.91	21.6	59.8	16.00								
		Out Control	4.3	6.90	21.9	55.2	1.68	840	0.019	4	4	5	10	7	
		Out Sand	5.0	7.03	22.0	60.3	1.80	715	0.023	8	12	4	7	4	
		Out Treated	4.7	7.21	22.1	61.3	1.46	1965	0.008	17	12	20	17	11	

Table A-1: Data collection table (Continued).

Date	Day	Sample Origin	D.O. (mg/L)	pH	Temp. (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Time for head volume to drop from 900 mL to 600 mL (s)	Falling head based hydraulic conductivity (cm/s)	*E. coli* Plate Counts					Notes:
										1	2	3	4	5	
12/2/11	24	Influent	9.2	6.83	20.4	51.1	14.00			274	293	305	302	292	
		Out Control	5.0	6.88	20.9	52.9	1.66	790	0.021						
		Out Sand	5.1	7.11	20.9	57.2	1.81	845	0.019						
		Out Treated	5.5	7.22	21.1	60.7	1.28	3410	0.005						
	24S	Out Control	7.0	7.74	21.0	51.7	2.67			670	670	660	580	610	Special sample collected for short residence time test.
		Out Sand	6.7	7.81	20.9	57.0	2.51			270	240	260	330	320	
		Out Treated	6.8	7.73	21.1	55.5	2.64			380	390	380	370	390	
12/3/11	25	Influent	8.7	6.88	18.7	30.9	16.90								
		Out Control	4.3	6.99	17.9	56.2	4.49	810	0.020	17	5	6	8	9	
		Out Sand	3.9	7.19	18.9	60.3	1.54	835	0.019	8	9	18	13	10	
		Out Treated	5.4	7.25	18.3	61.6	1.38	4370	0.004	21	17	18	14	20	
12/4/11	26	Influent	8.8	6.98	17.9	53.2	7.06			282	277	273	256	260	
		Out Control	5.0	7.10	18.6	58.6	1.39	800	0.020						
		Out Sand	4.5	7.32	18.6	61.8	1.15	1080	0.015						
		Out Treated	5.6	7.43	18.9	64.9	1.03	5180	0.003						
12/5/11	27	Influent	8.5	6.86	18.1	59.5	9.42								
		Out Control	4.8	6.91	18.4	55.1	1.09	800	0.020	6	7	4	9	9	
		Out Sand	4.0	6.99	18.4	59.6	1.08	1130	0.014	10	10	22	17	19	
		Out Treated	5.9	7.27	18.4	65.3	0.91	8580	0.002	15	16	8	13	13	

Table A-1: Data collection table (Continued).

Date	Day	Sample Origin	D.O. (mg/L)	pH	Temp. (°C)	Conductivity (µS/cm)	Turbidity (NTU)	Time for head volume to drop from 900 mL to 600 mL (s)	Falling head based hydraulic conductivity (cm/s)	*E. coli* Plate Counts					Notes:
										1	2	3	4	5	
12/6/11	28	Influent	8.8	6.96	17.3	69.5	4.87			206	187	253	217	295	Surface cleaned for heat-treated BSF.
		Out Control	4.6	6.90	17.8	59.4	1.37	825	0.020						
		Out Sand	4.4	7.01	17.7	61.4	1.09	930	0.017						
		Out Treated	4.0	7.02	17.9	60.6	1.37	655	0.025						
12/7/11	29	Influent	8.2	6.81	17.5	54.8	5.53								
		Out Control	4.2	6.83	17.5	57.8	1.16	825	0.020	20	30	19	26	37	
		Out Sand	4.1	6.95	17.5	60.8	1.02	885	0.018	10	9	11	14	23	
		Out Treated	4.0	7.04	17.5	60.4	1.08	855	0.019	60	42	50	61	70	
12/8/11	30	Influent	8.0	6.98	18.4	62.5	7.57			245	262	258	276	243	
		Out Control	4.6	7.04	19.3	63.5	0.94	950	0.017						
		Out Sand	4.0	7.14	19.2	63.9	0.92	720	0.023						
		Out Treated	4.5	7.12	19.3	62.9	1.08	1170	0.014						
12/9/11	31	Influent	8.4	6.80	18.9	47.0	4.95								
		Out Control	3.1	6.87	19.4	60.6	1.14	1065	0.015	16	15	11	10	10	
		Out Sand	3.8	7.02	19.4	65.7	1.05	965	0.017	26	27	31	16	29	
		Out Treated	3.5	7.03	19.4	66.3	1.06	2565	0.006	51	33	39	53	54	

Appendix K: Apparatus Photographs

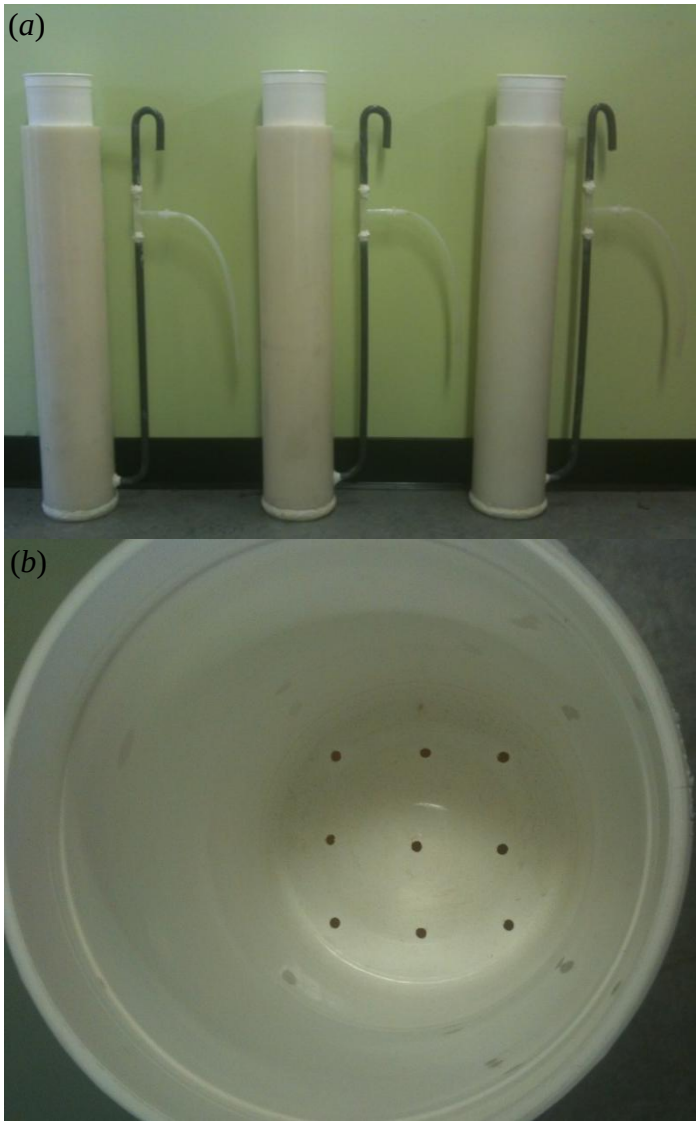

Figure A-1: Side-view of bench-scale Biosand filters (*a*) and top-view of the diffusion bucket (*b*).